OPTIMIZATION OF ANTIMICROBIAL THERAPY FOR GRAM-POSITIVE BACTERIAL INFECTIONS IN CHILDREN USING A TRANSLATIONAL PHARMACOLOGICAL APPROACH

This thesis is submitted in accordance with the requirements of The University of Liverpool for the degree of Doctor in Philosophy by

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Disclaimer

The data in this thesis is a result of my own work. The material collected for this thesis has not been presented nor is currently being presented, either wholly or in part for any other degree or other qualification. All of the research, unless otherwise stated, was performed in the Department of Molecular and clinical Pharmacology, Institute of Translational Medicine, University of Liverpool. The clinical PK study was conducted in Alder Hey Children´s NHS Foundation Trust and in the Neonatal Unit at The Liverpool Women´s NHS Foundation Trust, in Liverpool.

All other parties involved in the research presented here, and the nature of their contribution, are listed in the Acknowledgements section of this thesis.

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Dedication

To all the children and families that generously contributed to this research

To my parents and sister for their unconditional love and endless support

To Jesús, for his patience and contagious strength

"Primum, non nocere"

First, do not harm

Hippocrates

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Publications

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Abbreviations

ABPI UK Association of the British Pharmaceutical Industry

AHFT Alder Hey NHS Children´s Foundation Trust

AIC Akaike Information Criterion

AUC Area under the concentration-time curve

BIC Bayesian Information Criterion

BNFC British National Formulary for Children

BSI Bloodstream Infection

CDC Centre for Diseases Control

CFU Colony Forming Units

CL Clearance

CLABSI Central line-associated bloodstream infection

CLSI Clinical and Laboratory Standards Institute

C_{max} Maximum Concentration

C_{min} Minimum Concentration

CoNS Coagulase-negative Staphylococci

CRBSI Central line-related bloodstream infection

CRF Case Report Form

CRP C-reactive protein

CTIMP Clinical Trial of an Investigational Medicinal Product

CTSA Clinical Trial Site Agreement

CVC Central venous catheter

ECOF Epidemiological cut-off value

ELF Epithelial lining fluid

EMA European Medicines Agency

EUCAST-European Committee on Antimicrobial Susceptibility Testing

f free

FPIA Fluorescence Polarization Immunoassay

GA Gestational age

GFR Glomerular Filtration Rate

GISA/GRSA Glycopeptide intermediate/resistant *Staphylococcus aureus*

GRIP Global Research in Paediatrics

HFIM Hollow Fibre Infection Model

HIC High-income countries

HPLC High Performance Liquid Chromatography

IDSA Infectious Diseases Society of America

IMM Interacting multiple model

IQR Interquartile range

ke constant of elimination

kg kilogram

L Litre

LBW Low birth weight

LC/MS-MS Liquid Chromatography Tandem Mass Spectrometry

LD Loading Dose

LMIC Low and middle-income countries

LOS Late onset sepsis

LWH Liverpool Women´s NHS Hospital

MALDI-TOF Matrix-assisted Laser Desorption/Ionization-Time-of-Flight

MCRN Medicines for Children Research Network

mg milligram

MH Müller-Hinton

MHRA Medicines and Healthcare Products Regulatory Agency

MIC Minimum Inhibitory Concentration

mL millilitre

MM Multiple model

MRSA Methicillin-resistant *Staphylococcus aureus*

NHS National Health System

NICU Neonatal Intensive care unit

NONMEM Non-linear mixed effects modelling

PAP-AUC Population analysis profile-under the curve

PBP Penicillin binding protein

PBS Phosphate buffered saline

PD Pharmacodynamics

PIA Polysaccharide intercellular adhesin

PK Pharmacokinetics

PMA Post-menstrual age

PNA Post-natal age

PTA Probability of Target Attainment

R&D Research and Development

REC Research Ethics Committee

SCV Small colony variant

SD Standard Deviation

SPERA Solid-phase enzymatic receptor assay

SSP Staphylococcal surface proteins

SSTIs Skin and soft tissue infections

T> MIC Time of the dosing interval that the concentration is above the MIC

TDM Therapeutic Drug Monitoring

TSST Toxic shock syndrome toxin

VAP Ventilator-associated pneumonia

$V_{d/C}$ Volume of distribution

VISA/VRSA Vancomycin intermediate/resistance *Staphylococcus aureus*

VLBW Very low birth weight

WHO World Health Organization

µL microliter

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ABSTRACT: "Optimization of Antimicrobial Therapy for Gram-positive bacterial infections in children using a translational pharmacological approach"

Nosocomial bloodstream infection (BSI) is the most common type of hospital-acquired infection in paediatric patients and a major cause of morbidity and mortality worldwide. Methicillin-resistant staphylococci (CoNS and MRSA) are a leading cause of hospital-acquired neonatal sepsis and BSI. Glycopeptides (vancomycin and teicoplanin) constitute the current mainstay of therapy. There is limited antimicrobial PK-PD data available for neonates and children and optimal drug exposures resulting in maximal efficacy and suppression of resistance are not known. A translational pharmacological approach can be used to build the evidence required to optimize the current use of antimicrobial therapy in children.

Pre-clinical experimental (*in vitro* and *in vivo*) and clinical PK-PD work was conducted throughout this thesis to improve our understanding of the PK-PD relationships of vancomycin and teicoplanin against CoNS and MRSA. The *in vitro* HFIM defined the relevant PD indexes and free drug exposures associated with maximal bacterial killing and suppression of resistance. The *in vivo* models (a rabbit central-line associated BSI and a mouse neutropenic thigh infection model) validated the *in vitro* findings. CRP concentrations were incorporated as an *in vivo* PD input. A clinical PK study of teicoplanin in 57 patients (neonates, infants and older children) was conducted and the population PK parameters estimated. PK-PD modelling techniques were used to analyse the PK-PD data and bridge the experimental results to human patients by means of Monte Carlo (MC) simulations.

Vancomycin and teicoplanin displayed a concentration-dependent pattern of activity. An AUC:MIC ratio was associated with maximal antibacterial activity and suppression of resistance. Based on MC simulations, the probability of the *in vivo* target attainment with currently used teicoplanin dosage regimens results insufficient to treat a majority of patients with MRSA infection. High teicoplanin PK variability was identified in children. Weight, age and renal function were the best explanatory covariates of PK variability. Wider drug exposure distribution is observed in the paediatric population with respect to adults. A patient-tailored TDM approach with the aid of a Bayesian feedback adaptive control tool is required to ensure individual patients achieve optimal drug exposures in a precise and safe manner.

The defined pre-clinical optimal targets of exposure for vancomycin-CoNS and teicoplanin-MRSA now need to be prospectively evaluated in patients. Currently used teicoplanin dosage regimens in both, adults and children, may be insufficient to treat a high proportion of patients with serious MRSA infection. Current EUCAST clinical breakpoint may need to be revised for teicoplanin against MRSA. The current strategy of using teicoplanin fixed population-based antibiotic regimens results in a wide range of drug exposures in neonates and children. An individualised dosing and TDM approach can ensure optimal target attainment at the individual level and in real-time.

Chapter 1

General Introduction

1.1- OVERVIEW

Gram-positive bacterial infections are a leading cause of infectious mortality and morbidity in hospitalised children (H Wisplinghoff et al. 2003). In addition, the increasing prevalence of antimicrobial drug resistance or decreased susceptibility to current treatment regimens is a threat to public health worldwide (Sader et al. 2013; Ahlstrand et al. 2011). There are few antibiotics in the developmental pipeline and there is a renewed interest in investigating and optimising the use of well-established antibiotics (European Medicines Agency 2011; Ruggieri et al. 2014). However, insufficient PK/PD data is available for children and current dosage recommendations are outdated and lacking evidence.

There is an urgent need to develop evidence-based dosing regimens to treat Gram-positive bacterial infections that are efficacious, with reliable pharmacodynamics in children, as well as a favourable side-effect profile. Innovative PK/PD techniques can be used to identify antimicrobial regimens that result in successful bacterial killing, suppression of antimicrobial resistance and minimal drug toxicity. PK/PD relationships established in pre-clinical studies are predictive of therapeutic responses in humans (Craig 2014). Furthermore, the development of software to aid in the individualization of dosing to treat these infections represents a potential highly valuable tool to improve the clinical outcomes (Hope et al. 2013).

The overall “hypothesis” of this work is that innovative PK/PD techniques can be used to identify regimens of antimicrobials that are associated with maximal antibacterial killing and prevention of resistance in neonates and children with severe (hospital-acquired) Gram-positive bacterial infections, particularly bloodstream infections (BSI).

1.2- EPIDEMIOLOGY AND BURDEN OF GRAM-POSITIVE BACTERIAL INFECTIONS IN NEONATES AND CHILDREN: FOCUS ON STAPHYLOCOCCAL INFECTIONS

1.2.1- Epidemiology and burden of Gram-positive bacterial infections in neonates and children

Bacteraemia in children is a potentially life-threatening condition, which obliges immediate and effective antimicrobial therapy (Sharland 2007). Three of the most important influences on bacteraemia incidence are age, vaccination coverage and exposure to invasive procedures. For instance, higher rates are reported in younger children, infants and neonates, reflecting the relative immaturity of their immune system, as well as the increasingly broader use of medical devices in pre-term and term neonates (Michael Cohen-Wolkowiez. et al 2009).

Neonates have deficiencies in both the innate and adaptive immunity, and in the interaction between the two systems (Wynn et al. 2009). For instance, they have decreased complement components, reduced expression of some antimicrobial proteins and diminished production of type I interferon and Th1 polarizing cytokines (with a Th2-skewed response). They present with quantitative and/or qualitative impairments in neutrophil, monocyte, macrophage and dendritic cell function (Levy 2007). There exists also differences between pre-term and healthy term neonates with respect to absolute cell counts of B cells and the frequency of regulatory T cells, more pronounced in the earliest pre-term neonates (GA< 30 weeks)(Huenecke et al. 2016). All these deficiencies in cellular and non-cellular immune responses lead to an impaired phagocytosis and decreased pathogen clearance in neonates as compared to older children and adolescents.

Nosocomial bloodstream infection (BSI) is the most common type of hospital-acquired infection in paediatric patients and a major cause of morbidity and mortality worldwide (H Wisplinghoff et al. 2003). A report from a bacteraemia surveillance study in England (1992-2005, General Practice Research Database and Hospital Episode Statistics), in children aged under five, revealed that in 1992, around a 50% of bacteraemia were due to Gram-positive bacteria (Sharland 2007). However, this increased to over 75% in 2003 with Coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* (SA) becoming the leading significant bacterial pathogens reported, as shown in **figure 1.1** (Sharland 2007). Another report from the Health Protection Agency (HPA) database described more recently the trends in the incidence of bacteraemia in England and Wales across the whole child age range (1 month-15 years old)(Henderson et al. 2010). Over a 10-year period (1998-2007), total annual reports of bacteraemia increased with a mean 6.5% rise per year and in 2007, just over half of the cases were accounted for by 4 groups of Gram-positive microorganisms: CoNS (28%), SA (10%), non-pyogenic streptococci (9%) and *Streptococcus pneumoniae* (7%) (Henderson et al.2010).

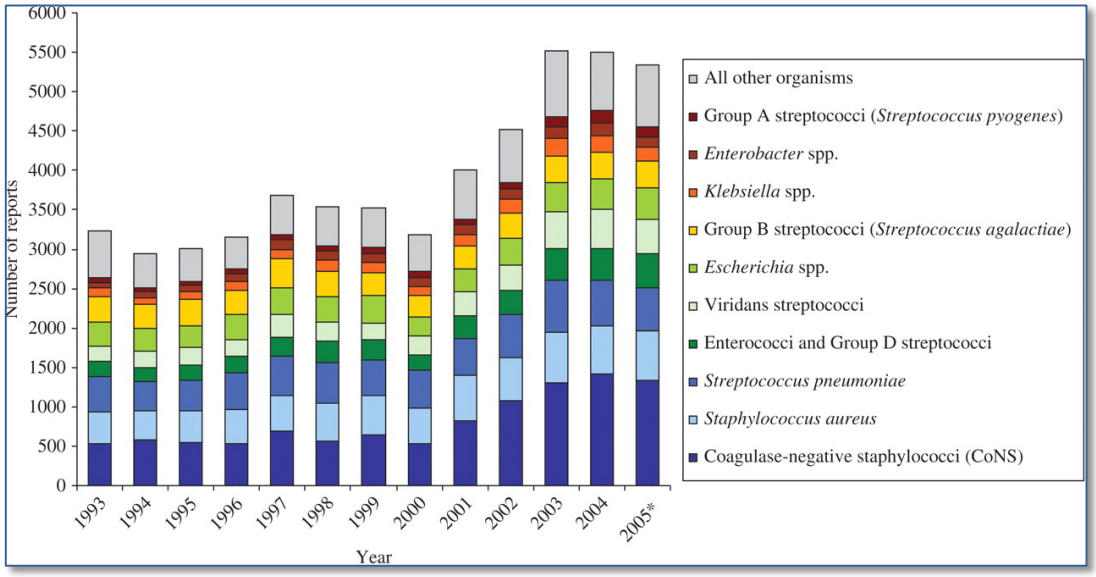

Figure 1.1 - Bacteraemia in children < 5 years old in England and Wales, 1992-2005. *2005 data published as provisional (Sharland 2007).

On a more global basis, a report from a prospective evaluation involving 49 US hospitals over a 6-year period (1995-2001), showed that in children up to 16 years old, a 65% of the isolates recovered from BSI were Gram-positive bacteria, 24% Gram-negative, 11 % fungi and 0.14% anaerobes. Among those, CoNS accounted for almost half of all nosocomial BSI (43%), followed by enterococci, *Candida spp*., and SA (9% each). Interestingly, when age groups were compared, the proportion of CoNS isolates diminished from 46% in < 1 year olds to 31 % in > 5-year-olds, whereas the proportion of SA and *Enterococcus sp*. increased from 8 and 9%, respectively in the younger children to 12% in the older paediatric patients (statistical significance of these differences was not reported). According to the predisposing factors of BSI, intravascular devices were the most common ones. In this multi-centre study, the crude mortality rate of nosocomial BSI during hospitalization was 14 % (475 /3452 patients) (H Wisplinghoff et al. 2003).

In countries with limited resources, the trends in the aetiology of paediatric BSI appear different to those stated in high-income countries (HIC). Although limited reports are available, several studies have shown a predominance of Gram-negative bacterial infections in hospitals. For instance, a retrospective 4-year study from a Ghanaian tertiary referral teaching hospital, showed that Gram-negative bacteria was most frequently isolated in BSI in children and infants than Gram-positive bacteria (57.5% and 54.4% *versus* 40.7 and 45.1% respectively) (Obeng-Nkrumah et al. 2016). Notably, neonates were excluded from this study. Furthermore, a systematic review of community-acquired neonatal and infant sepsis in low and middle-income countries (LMIC), including the aetiology and antibiotic resistance patterns of neonatal sepsis, was conducted in order to estimate the efficacy of WHO antibiotic guidelines. Nineteen studies were identified from 13 countries from Asia and Africa. Among neonates, SA, *Klebsiella spp*. and *E. coli* accounted for 55% of all isolates. In infants outside the neonatal period, the most frequently isolated pathogens were SA, *E. coli*, *Klebsiella spp.*, *Salmonella spp*. and *Streptococcus pneumoniae*, accounting for 59% (Downie et al. 2013). In summary, the available evidence underlines a higher prevalence of Gram-negative than Gram-positive bacteria in the aetiology of BSI in LMIC than in HIC and many epidemiological factors could be involved in this microbiological shift. For instance, there is increasing evidence that clinical pathogens vary incidence density with temperature (Schwab et al. 2014). Nonetheless, similarly to the reports from HIC, SA was the most common isolated causative pathogen overall in these studies.

In hospital morbidity rates of BSI and sepsis remain high, particularly among preterm infants, while contributing to adverse and potentially disabling neurodevelopmental outcomes. This risk of morbidity is inversely proportional to

gestational age (GA) at birth (Stoll et al. 2010). The persistent inflammatory state of infection has been reported as the main *noxa* on the developing brain, which is not directly related to the specific pathogen (Adams-Chapman & Stoll 2006; Mitha et al. 2013).

A significant persistent challenge for the clinician is how to effectively treat Gram-positive bacterial infection given the increasing diversity of drug-resistant pathogens and the limited treatment options. Thus, it is important to find ways to optimally treat the most common causes of hospital-acquired infection, namely Gram-positive BSI and sepsis, in these vulnerable populations in order to eradicate the causative microorganism and minimize inflammation.

1.2.2- Coagulase-negative Staphylococcal Infections

The pathogenic potential of CoNS became accepted by the end of 1980s, but at the time most of the underlying molecular mechanisms were unknown. Modern and molecular-phenotypic methods have expanded our knowledge on its clinical significance. As opportunistic microorganisms, colonization of different parts of the skin and mucosa, including the gastro-intestinal tract of the host, is the key source of invasive staphylococcal infections (Soeorg et al. 2013). Demographic and medical developments have contributed to its increasing health burden. Like other nosocomial pathogens, increasing rates of resistance are even a greater problem for CoNS than for SA. Although CoNS possess fewer virulence properties than SA, host susceptibility plays a main role in the pathogenesis, and therapeutically, CoNS are challenging for three reasons: 1) the uncertainty with respect to the clinical distinction between significant infection *versus* contamination, 2) the great proportion of methicillin-resistant strains and 3) the decreasing susceptibility to

glycopeptides (von Eiff et al. 2002; Butin et al. 2015), currently the mainstay of therapy.

Taxonomy and classification

CoNS represent a heterogeneous group within the genus *Staphylococcus* that is not based on phylogenetic relationships. A simplified but useful and widely accepted scheme for the classification based on clinical and diagnostic approaches is used in human medicine. That is, staphylococci are divided into coagulase-positive, almost exclusively represented by SA, and coagulase-negative- CoNS-, initially viewed as non-pathogenic. As of 2014, the genus *Staphylococcus* consists of 47 species and 23 subspecies. Of these, 38 are recognized as CoNS and 13 of them, known to colonize humans (Venkatesh et al. 2006a). The clinically defined " S. epidermidis group" comprises *S. epidermidis* and *S. haemolyticus* as the most prevalent species, as well as *S. capitis*, *S. hominis*, *S. warneri and S. lugdunensis* (novobiocin susceptible strains) implicated in native and prosthetic valve endocarditis, foreign body infections, osteomyelitis and in infections among immune-suppressed patients (Becker et al. 2014; Ruhe et al. 2004; Elamin et al. 2015). The novobiocin resistant strains include *S. saprophyticus* and *S. xylosus* with the former being a well-known cause of urinary tract infections in immune-competent women (Widerström et al. 2007). Recently, Lamers et al proposed a refined classification of 6 species groups and 15 cluster groups based on molecular data of the genus *Staphylococcus*. For a complete overview of the phylogenetic classification of staphylococcal species and sub-species extended by the diagnostic characteristics, as proposed by Lamers *et al*., see **figure 1.2** (Lamers et al. 2012)**.**

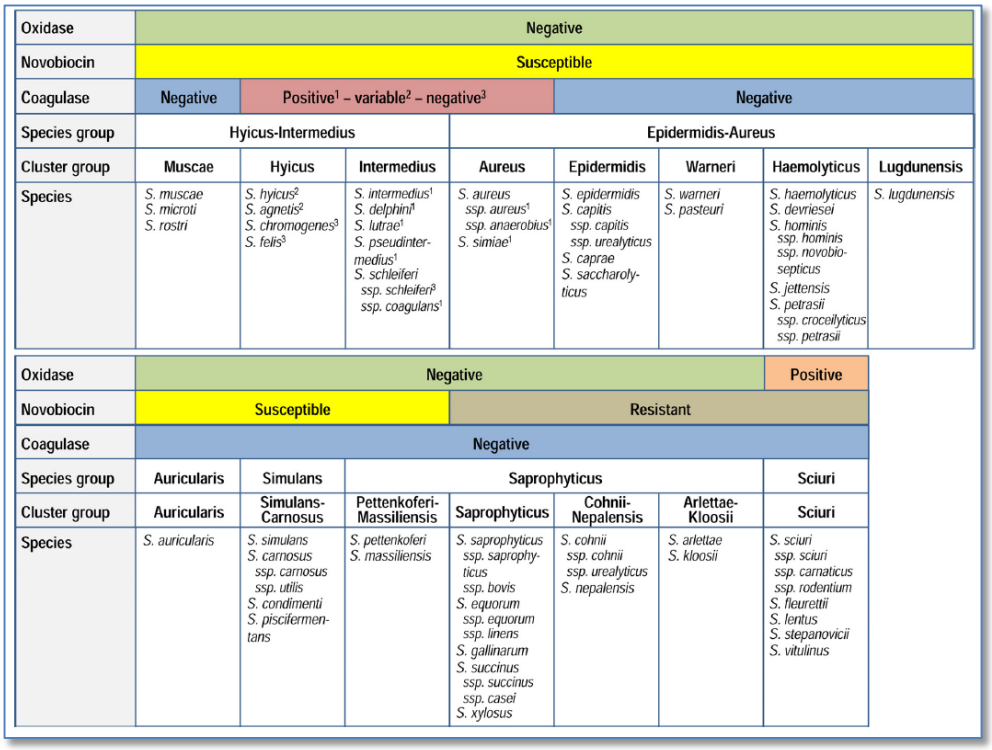

Figure 1.2-Phylogenetic classification of staphylococcal species and sub-species extended by the diagnostic characteristics, as proposed by Lamers *et al*.(Lamers et al. 2012) taken from the review by Becker *et al* (Becker et al. 2014).

Pathogenesis and virulence of CoNS

Most of the research conducted into CoNS virulence factors has been mainly focused on *S. epidermidis*, particularly in reference to adhesion and biofilm formation characteristics in foreign-body/device-associated infection (Veenstra et al. 1996; Heilmann et al. 1997).

Four main steps compose this process:

1) Attachment to unmodified polymer surface.

Bacterial adherence to polymer surfaces generally depends on bacteria cell surface characteristics and on the nature of the biomaterial. Initial attachment to unmodified polystyrene is mediated, at least in part, by two antigenically related staphylococcal surface proteins (SSP-1 and SSP-2)(Veenstra et al. 1996). The

surface-associated autolysin AtLE is also involved, not only in this initial attachment, but also binds to the extracellular matrix protein vitronectin in the second step of the process (Heilmann et al. 1997). In addition to proteins, a capsular polysaccharide/adhesin (PS/A) has been associated with adhesion and biofilm formation in a rabbit model of endocarditis caused by *S. epidermidis*. Furthermore, immunisation with this PS/A has resulted in protection against infection (Shiro et al. 1994).

2) Attachment to polymer surface coated with extracellular matrix proteins.

This step can be achieved through transcutaneous migration and/or haematogenous seeding from a distant site (von Eiff et al. 2002). After insertion of the medical device, the polymer material rapidly becomes coated with plasma and extracellular matrix proteins such as fibrinogen, fibronectin, vitronectin, trombospondin and von Willebrand factor (Herrmann et al. 1988; Herrmann et al. 1997).

At later stages of infection, these proteins deposited in polymer-coated surfaces facilitate the colonisation from further staphylococci through specific cell-surface-receptor. For instance, adherence of clinical isolates of coagulase-positive and negative staphylococci to biomaterials is enhanced by surface-bound fibronectin, particularly in SA while this varies among *S. epidermidis* strains (Herrmann et al. 1988). Besides proteins, cell-wall teichoic acid is also involved in the adherence of *S. epidermidis* to proteins, such as fibronectin (Hussain et al. 2001).

3) Proliferation and accumulation in multi-layered cell clusters. Biofilm formation.

This helps the microorganism to evade the host mechanisms of defence and the antibiotic effect. After adherence to the biomaterial surface, the Staphylococci multiply and accumulate as multi-layered cell clusters. This process involves intercellular adhesion. The importance of *S. epidermidis* polysaccharide intercellular adhesin (PIA) has been shown *in vivo*. A PIA-negative mutant was significantly less virulent than the isogenic wild-type strain in a mouse model of subcutaneous foreign body infection and in a rat model of central venous catheter-associated infection (Rupp, Ulphani, Fey, Bartscht, et al. 1999; Rupp, Ulphani, Fey & Mack 1999). The *ica* ABC genes mediate cell clustering and PIA synthesis in *S. epidermidis.* An additional open reading frame (*ica*D) has been identified (Heilmann et al. 1996; Gerke et al. 1998). The *ica* ADBC operon mediates biofilm accumulation, PIA production and haemagglutination (Fey et al. 1999). It has been shown in strains of *S. epidermidis* obtained from blood cultures of patients with prosthetic device-associated sepsis that there was a strong association between pathogenesis and both, biofilm formation and the presence of the *ica* gene cluster. As opposed, skin and mucosal isolate colonisers were usually biofilm-negative and lacked the *ica* genes (Ziebuhr et al. 1997; Cherifi et al. 2013). Furthermore, PIA inhibits phagocytosis and killing by polymorphonuclears (Vuong, Kocianova, Voyich, et al. 2004).

4) Biofilm detachment and metastatic seeding.

Finally, upon biofilm maturation, individual or clusters of bacteria may disaggregate and disperse via the bloodstream. Then, further sites in the body may be colonised leading to the metastasis of infection. This disintegration may be mediated

by several mechanisms, such as extracellular enzymes and the small peptides called phenol-soluble modulins (PSMS)(Mehlin et al. 1999; Dubin et al. 2001; Oleksy et al. 2004).

Other potential virulence factors in *S. epidermidis* are several proteases, toxins (e.g δ-toxin), production of lantibiotics that interfere with other bacteria on skin and mucous membrane colonisation and mechanisms of iron acquisition from the host (Chamberlain & Brueggemann 1997; McKevitt et al. 1990; Kupke & Gotz 1996; Modun et al. 1998). In addition to the *ica* operon, another virulence-associated mobile genetic element is the arginine catabolic mobile element (ACME), which comprises 2 gene clusters: a six-gene *arc* operon that encodes an arginine deiminidase and the *opp3* that encodes an oligopeptide permease system (Diep et al. 2008). The role of ACME has not been completely determined, but various studies have shown that increases the ability of *S. epidermidis* strains to colonize skin and mucosa, as well as enhances haematogenous dissemination to target organs (Du et al. 2013; Diep et al. 2008).

Host response interactions

Opsonophagocitosis is the primary host defence mechanism against CoNS infection (Krediet et al. 1998). CoNS biofilm exerts immunomodulatory properties while stimulating the production of prostaglandins by monocytes, which inhibits T-cell proliferation and function (Stout et al. 1992). In the last decade, research has shown that increased levels of biofilm production are conversely associated with a decreased incidence of tissue invasion, and lower C-reactive values have been demonstrated in neonatal CoNS sepsis caused by biofilm-positive *versus* biofilm-negative isolates (Vuong, Kocianova, Yao, et al. 2004; Klingenberg et al. 2005). This research supports the theory that this could limit the immune system to counteract

the infection and points to host-response evasion of CoNS (within the biofilm) as the main virulence factor. Conversely, a strong association between biofilm production and both phenotypic and genotypic antibiotic resistance has also been shown (Klingenberg et al. 2005).

Nonetheless, there is a paucity of data that links virulence factors, antibiotic resistance and clinical outcomes in CoNS infection, as opposed to SA infection.

Clinical significance of Coagulase-negative Staphylococcal infections in the child

CoNS infection is the most common cause of laboratory confirmed blood stream infection (LCBSI) in the paediatric and neonatal intensive care setting (National Nosocomial Infections Surveillance (NNIS) system report, 2001; Elward et al. 2005). It is frequently isolated in patients who are low and very low birth weight and/or pre-term infants, immunosuppressed (e.g febrile neutropenia in oncologic patients), have significant burns or have indwelling intravascular devices, ventricular shunts, peritoneal catheters or other implanted/inserted medical devices (Vergnano et al. 2011; Stoll et al. 2002; El-Mahallawy et al. 2009). In the intensive care setting, these infections represent an important cause of global morbidity, prolonged hospital stay and mortality, as well as considerable increased costs in patients who develop nosocomial BSI in comparison with non-infected patients with similar underlying conditions and the same number of ventilator days (Elward et al. 2005).

Paediatric oncologic patients, bone marrow transplant recipients and children with burns are at high risk for acquiring CoNS bloodstream infections (Miedema et al. 2013; Santucci et al. 2003). In a national surveillance study comprising England and Wales over a 10-year period in children aged 1month-15 years old, CoNS bacteraemia represented a 28% of the organisms causing bacteraemia in hospitalized children (Henderson et al. 2010).

CoNS infection in the neonate and infant

Neonatal sepsis is associated with significant morbidity and mortality (Levit et al. 2014; Stoll et al. 2010). It is generally classified as early-onset or late-onset sepsis. Early onset reflects infection with organisms acquired before or during birth. Late onset occurs more than 72 h after birth and reflects nosocomial infection (Hornik et al. 2012). The incidence of neonatal sepsis is inversely proportional to gestational age and birth weight. Very low birth weight (VLBW < 1500 grams) represents 5 % of all births. Among VLBW neonates, culture-proven early onset sepsis is seen in 2% of neonates and late-onset in 25% (Hornik et al. 2012).

CoNS are the most common cause of late-onset sepsis, being responsible for 30-54% of all cases in some studies (Vergnano et al. 2011; Stoll et al. 1996; Stoll et al. 2002). Furthermore, central-line associated infections (CLABSI) are the most common risk factor for CoNS sepsis, particularly in the neonatal intensive care unit (NICU)(Milstone et al. 2013).

Morbidity is a major issue due to the potential impairment on brain development (e.g white matter injury due to inflammation, hypoxia and ischaemia). Developmental delay and disability is increased among children that suffer from recurrent infection during the neonatal period (Glass et al. 2008).

1.2.3- Staphylococcus aureus (SA) infections

Staphylococcus aureus (SA) colonises the skin and mucosa of about 30% of healthy humans, however, as opportunistic microorganism, it continues to be an important cause of serious health-care-associated and community-acquired infections worldwide (Wertheim et al. 2005). In the UK alone, around 9,100 cases are reported voluntarily each year with an associated mortality of about 29% (Public Health of

England 2014)(Wyllie et al. 2006). Its oxacillin-resistant form (MRSA) has been the most important cause of antimicrobial resistant healthcare-associated infection globally.

Although current surveillance data from US and Europe indicate a decrease in the incidence of healthcare-associated MRSA invasive infections compared to baseline data, MRSA remains a significant pathogen and a public health priority with high associated morbidity and mortality (Centers for Disease Control and Prevention 2014; European Centre for Disease Prevention and Control 2015). This may be due to the intrinsic virulence of SA, to the sub-optimal antimicrobial therapy with important delays in the administration and to the increasing multi-drug resistance of strains displayed in the last decade. In addition, the evidence supporting an optimum management of SA bacteraemia is still poor and clinical practice is driven mainly by the results of observational studies as well as by educated guess.

Taxonomy and classification

SA belongs to the genus *Staphylococcus* and it represents almost exclusively the coagulase-positive diagnostic sub-group. The refined phylogenetic-diagnostic classification of staphylococcal species and subspecies proposed by Lamers *et al*. (Lamers et al. 2012) shows its location within the genus. See **Figure 1.2.**

Molecular-based epidemiologic studies have revealed that 5 major MRSA clones account for near a 70% of hospital MRSA isolates worldwide (Iberian, Brazilian, New York-Japan, Hungarian and Paediatric)(Oliveira et al. 2002). The epidemiological factors that contributed to the dissemination of only a few MRSA clones are not yet well elucidated. In Europe, MRSA isolates that cause invasive infections are less diverse than invasive MSSA isolates and MRSA *spa* types (small

region of one gene typed) have a predominant geographic distribution within distinct clusters of health networks (Grundmann et al. 2010).

Pathogenesis and virulence of SA

The ability of SA to cause invasive and/or severe infection depends upon a large variety of virulence factors to enable adherence, aggression, invasion, persistence and/or evasion of the immune system, often located on mobile genetic elements that are transferred horizontally through the SA population. Adherence to surfaces and phases of biofilm formation is a common first critical event in establishing colonization and/or infection among staphylococci. The additional phases of the process are shared with the CoNS microorganisms as detailed above.

A number of genes both in the SCC-*mec* region and outside this region, have been identified in complicated infections, including bacteriophage-encoded genes, such as regulatory factors and autolysins, with likely roles in tissue adhesion and biofilm formation (Gill et al. 2011). In addition, molecular connections between virulence and antibiotic resistance have been found with the newly identified *psm-mec* gene that encodes for a phenol soluble modulin (α-type PSM) with significant cytolytic and immune evasion capacity within the SCC*mec* region, so far believed to be encoded in the core genome of the bacterium (Queck et al. 2009).

Compared to CoNS, SA is characterized by high aggressiveness that involves not only a wide array of cytolytic toxins, but also pyrogenic toxin superantigens (PTSAgs) and exfoliative toxins, such as the toxic shock syndrome toxin 1 (TSST-1) that contribute to poorer clinical outcomes and mortality in SA bacteraemia (Maeda et al. 2016). A further strategy of SA to evade the host response are the small-colony variants (SCVs), a well described adapted phenotype of SA that confer intracellular persistence and antibiotic-refractory infections (Tuchscherr et al. 2010). Several lines

of research are currently centred in further understanding the association between virulence factors, pathogenesis and antimicrobial resistance in SA infections with the aim of improving the existing control and therapy measures, including vaccination and disease prevention.

Clinical significance of SA infections in the child

The clinical spectrum of SA infection in children ranges from asymptomatic nasal carriage to skin and soft tissue infections (SSTIs), osteomyelitis, septic arthritis, infective endocarditis, bacteraemia, sepsis and death (Kaplan 2016; McNeil et al. 2016). Colonization is an important factor in the pathogenesis of infections caused by SA. Children are particularly susceptible to colonization with prevalence as high as 56.7% depending on the age group, being higher in the young infant population (Regev-Yochay et al. 2009; Williamson et al. 2016). They also present with a pattern of persistent colonization and constitute the main vectors in disseminating SA throughout the community and in the hospitals (Williamson et al. 2016). As opposed to CoNS, a single positive blood culture for SA should always be defined as clinically significant, given the intrinsic pathogenicity of SA and the high number and frequency of complications following SA bacteraemia (Naber et al. 2009).

SA infections in the neonate and infant

Invasive SA bloodstream infections in the neonate and infant, particularly in the pre-term infant, present with a wide range of serious complications: brain or visceral abscesses, meningitis, orbital cellulitis, osteomyelitis, septic arthritis, endocarditis, pneumatoceles and lung abscesses, septic ileus, septic shock and death (Healy et al. 2004). Interestingly, community-associated MRSA strains have also emerged in the past decade as an important cause of LOS in the NICU with even

greater morbidity and mortality than hospital-associated strains, despite appropriate therapy (Healy et al. 2004).

In addition, prospective neonatal data for VLBW infants (<1500 g) has showed that mortality from MRSA *versus* MSSA bacteraemia and/or meningitis is high with both MRSA (26%) and MSSA infections (24%). Thus, the authors recommend that in this special population, the efforts in infection prevention and control should not only be focused in MRSA but also in MSSA screening and isolation as they have equivalent morbidity and mortality (Shane et al. 2012).

1.3- TREATMENT, MANAGEMENT AND DRUG RESISTANCE PATTERNS OF STAPHYLOCOCCAL BLOODSTREAM INFECTIONS IN CHILDREN (CoNS AND SA)

1.3.1- Treatment and management of CoNS infections (bacteraemia)

Several factors need to be taken into account for the appropriate management of CoNS infections and these are: the species (*S.epidermidis* group vs *S.lugdunensis* vs *S.saprophyticus*), the site of infection, the patient´s immune status and the presence of an inserted medical device. Glycopeptide therapy, with vancomycin or teicoplanin, represents the mainstay of antibiotic therapy, given the high rates of methicillin resistance, 70-92% in some studies (Krediet et al. 2004). Co-trimoxazole, if the isolate is susceptible, or newer antibiotics such as daptomycin, linezolid or 5th generation cephalosporins also constitute possible alternatives. For MS-infections, β-lactamase-resistant penicillins or cephalosporins (1st or 2nd generations) can be appropriate.

Combination therapy of agents with cell wall activity and rifampicin has been shown to be synergistic, although there is no randomized and/or extensive clinical evidence to support this. For instance, the persistence of CoNS bacteraemia in neonates (≥5 days of positive blood cultures despite appropriate antibiotic therapy) has been shown to respond successfully to the addition of iv rifampicin to glycopeptides in small case series (Tan et al. 1993; Shama et al. 2002).

For infections caused by *S.lugdunensis* there is a paucity of controlled clinical data to inform its most effective antimicrobial therapy. β-lactamase-resistant penicillins and $1^{st}/2^{nd}$ generation cephalosporins might be appropriate in the empirical treatment. In the case of infective endocarditis by this pathogen, supportive therapy is also necessary (e.g valve replacement)(Liu et al. 2010). Likewise, the case of uncomplicated UTI caused by *S.saprophyticus* (subspecies *saprophyticus*) might be appropriately treated with co-trimoxazole, however, duration of therapy is considered to be more appropriate for 7 days rather than 3 days as per the case of other causative pathogens (Guay 2008).

In the context of foreign body and/or catheter related infections, the decision to remove the intravascular catheter or initiate empirical therapy while trying to salvage the device, is still a matter of debate and a therapeutic challenge, particularly in the paediatric and neonatal population. For example, a cohort study conducted in 148 infants with central venous catheter (CVC) and positive blood cultures caused by CoNS, the early removal of the CVC (within 3 days of diagnosis) and treatment with vancomycin alone, achieved eradication of bacteraemia. If early removal was not possible, eradication of bacteraemia was possible in 46% of neonates with vancomycin alone and late removal. If bacteraemia lasted longer than 4 days, eradication was not possible without CVC removal (Karlowicz et al. 2002a). In

another study, retrospective in nature, looking at 72 neonates with CoNS CLABSI, the successful treatment and salvage of the catheter was possible in 51.3% of patients. However, the longer the bacteraemia was (> 4 positive blood cultures), the greater the risk of having deleterious clinical outcomes (end organ damage and death)(Benjamin et al. 2001).

In order to save the CVCs, a systematic literature review reported the supportive role of associated antibiotic lock therapy for both preventing and treating CLABSIs (Segarra-Newnham & Martin-Cooper 2005). A prospective randomized controlled trial in 85 critically ill pre-term neonates, also showed the advantage of vancomycin lock therapy (25 μg/mL) as prophylaxis in reducing the incidence of CLABSI (Garland et al. 2005). In summary, biofilm formation associated to inserted medical devices, reduces the efficacy of antibiotics while increasing considerably their MICs due to their impaired penetration. If CVC removal is clinically possible and is performed early, a short course of antibiotic therapy (5-7 days) is sufficient, however, if the line is retained, longer systemic and lock therapy for 10-14 days should be provided (Mermel et al. 2009). Nonetheless, there is no preferred choice established due to the lack of controlled clinical data.

Finally, prosthetic joint infections caused by staphylococci require longer courses of therapy (2-6 weeks) after debridement and retention of the prosthesis, followed by longer oral courses. IDSA recommends either $1^{st}/2^{nd}$ generation cephalosporins or β-lactamase-resistant penicillins if MS-isolates, but vancomycin is the antibiotic of choice in the case of MR-staphylococci. Alternatives are daptomycin, linezolid or combination therapy with rifampicin (Mermel et al. 2009).

1.3.2- Treatment and management of SA bacteraemia

Currently, UK and US guidelines suggest that uncomplicated SA and MRSA bacteraemia (a positive blood culture and exclusion of endocarditis, no implanted prostheses, defervescence within 72 hours of therapy, negative follow-up blood cultures 2-4 days after initial set and no evidence of metastatic sites of infection) should be treated with vancomycin for a minimum of 14 days, and for 4-6 weeks if there is a deep infectious focus (Gould et al. 2009; Mermel et al. 2009; Liu, Bayer, Cosgrove, Daum, Fridkin, Gorwitz, Kaplan, Karchmer, Levine, Murray, Rybak, D. A. Talan, et al. 2011). There is strong evidence to suggest that prompt removal or drainage of medical devices/ infected foci improves outcome (Thwaites et al. 2011).

In children, duration may range from 2-6 weeks depending on source, presence of endovascular infection and metastatic foci of infection. Glycopeptide therapy, namely vancomycin and teicoplanin are the agents of choice. Data regarding the safety and efficacy of alternative agents in children are limited, although daptomycin 6-10 mg/kg/dose iv once daily may be an option. Clindamycin and linezolid may be considered but should not be used if there is concern of infective endocarditis or endovascular source. Finally, the decision to use combination therapy with rifampicin or gentamicin should be individualized, although the addition of gentamicin to vancomycin in adults is not recommended (Liu, Bayer, Cosgrove, Daum, Fridkin, Gorwitz, Kaplan, Karchmer, Levine, Murray, Rybak, D. A. Talan, et al. 2011).

1.3.3- *In vitro* susceptibility testing

Phenotypic approaches

Antimicrobial susceptibility testing for staphylococci is based on the reference methods of the Clinical and Laboratory Standards Institute (CLSI) and the European Union Committee for Antimicrobial Susceptibility Testing (EUCAST). Phenotypic-based approaches have reduced sensitivity and specificity for the determination of methicillin-resistance in CoNS and SA due to the hetero-resistance phenomenon. Applying cefoxitin as a disk diffusion test to grown cultures has overcome many of the associated issues.

Susceptibility testing for glycopeptides is method dependent. The standard method should be broth microdilution (Leclercq et al. 2013). In contrast, the disk diffusion method is unable to distinguish between the wild-type phenotype and those isolates with non *van*A-mediated resistance. GISA (glycopeptide-intermediate resistance SA) infections are not treatable with high doses of glycopeptides, therefore, the EUCAST reduced the resistance breakpoint to 2 mg/L in order to avoid GISA isolates being reported as susceptible. Thus, there is no difference in reporting GISA/VISA and GRSA/VRSA isolates. A modified population analysis profile-under the curve (PAP-AUC) has been described for reporting hetero-resistance in glycopeptides, but this is not practical in routine use, so several screening assays have been developed, particularly for SA, such as the macro-Etest method, also applied in CoNS (*S.epidermidis* and *S.haemolyticus*)(Ahlstrand et al. 2011).

Nucleic acid detection-based approaches

For the detection of MRSA for screening and surveillance purposes, there are rapid PCR-based methods, however, these do not exist for MR-CoNS. Nonetheless,

in-house PCR approaches exist for CoNS that cover the methicillin-resistance determination, species identification and for even biofilm formation gene detection (Iorio et al. 2011).

Recently, a rapid test to detect methicillin-resistance in SA through the PBP2a antigen has been introduced in a paediatric hospital for the rapid and accurate diagnosis of skin and soft tissue infections (Yu et al. 2016).

1.3.4- Antimicrobial resistance in Staphylococci to current treatment options

A continuous increasing loss of susceptibility towards most of the available antibiotics has been reported for CoNS over the past decades. CoNS are thought to represent an important reservoir of genetic elements, mobile by nature, that lead to resistance not only to β-lactams, but also to other antibiotic classes and may be transferred into medically significant staphylococci, both coagulase-negative and positive species.

In recent years, CLSI and EUCAST have reduced susceptibility breakpoints for CoNS (with the exception of *S. lugdunensis*) and SA, therefore, susceptibility categories of older studies may underreport resistance among CoNS populations (Clinical and Laboratory Standards Institute 2012; European Committee on Antimicrobial Susceptibility Testing 2015).

Resistance to β-lactamase activity

This phenotype is caused by penicillinases, which represent mainly plasmid-mediated (also chromosomally-mediated) staphylococcal β-lactamase encoded by the *blaZ* gene (Olsen et al. 2006). Penicillinases show specificity for penicillins and

act via hydrolysis of the β-lactam ring. Shortly after the introduction of the first penicillinase-stable penicillin as a therapeutic agent in the early 1960s, about a 10% of *S. epidermidis* isolates tested resistant to methicillin ("calbenin" or "staphcillin"). Subsequently, dramatic increases in the percentage of resistant isolates have been noted, particularly for the isoxazyl-penicillins and/or penicillinase-stable β-lactams, but also for ciprofloxacin, clindamycin, erythromycin, gentamicin, fusidic acid and rifampicin (Lyytikäinen et al. 1996; Kresken & Hafner 1999; Andrews et al. 2000). A significant correlation between % of methicillin resistance and usage of penicillinase-stable β-lactam agents has existed over the years (Lyytikäinen et al. 1996).

Nowadays, penicillin-susceptible *S. epidermidis* and *S. haemolyticus* occur very rarely (< 10%) among isolates recovered from hospitalized patients (Kresken et al. 2011). Testing for β-lactamase production in *Staphylococci spp.* is discouraged by the EUCAST due to this high prevalence of resistance to penicillins in most countries, as well as additional technical testing problems (Leclercq et al. 2013). Furthermore, the vast majority of clinical human CoNS isolates possess SCC*mec* (Staphylococcal Cassette Chromosome) elements as the main driver of resistance to β-lactams.

Resistance to β-lactams by expression of an additional penicillin-binding protein (PBP)

In staphylococci, the expression of an additional PBP, designated PBP2a or PBP2´, leads to complete β-lactam resistance (penicillins, most cephalosporins and carbapenems), with the only exception of the recently introduced cephalosporins with MRSA activity, such as ceftobiprole and ceftaroline. This PBP2a is encoded by the *mec*A gene, part of a mobile genetic element called Staphylococcal Cassette

Chromosome (SCC)*mec,* and shows reduced binding affinity for β-lactam antibiotics, in contrast to the intrinsic set of staphylococcal PBPS 1 to 4 (Katayama et al. 2000).

The molecular organization of this staphylococcal resistance to β-lactams is complex with a recognized increasing diversity of polymorphisms at the gene level within the SCC*mec* element and the large SCC family. To date, 11 types and several subtypes have been reported for the SCC*mec* element. In CoNS, SCC*mec* types III, IV and V, either alone or in various combinations, are the most prevalent types (Zong et al. 2011). Furthermore, there exists several *mec*A gene homologs, 32 unique alleles that clusters into 4 distinct branches (*mec*A, *mec*A1 and 2, *mec*B and *mec*C). The branch that contains the "classical *mec*A gene" involves the vast majority of methicillin-resistant CoNS (MR-CoNS) and the common MRSA lineages. The cassette (SCC*mec*) is constituted of three main elements: 1) the *mec* gene complex, 2) the *ccr* gene (cassette chromosome recombinase) complex and 3) the joining "junkyard" or J regions, each of those comprising additional virulence and resistance genes. To describe a MR-CoNS or MRSA strain in terms of its SCC*mec* composition, a complex nomenclature has been established by the International Working Group on the Classification of Staphylococcal Cassette Chromosomal Elements (International Working Group on the Classification of Staphylococcal Cassette Chromosome 2009).

The percentage of MR-CoNS has continuously increased with prevalence of 80% or more (Kresken et al. 2011; Sader & Jones 2012; Gordon et al. 2012). In addition, MR-CoNS are, in general, more often multi-resistant than methicillin (oxacillin)-susceptible isolates (Natoli et al. 2009).

Resistance to glycopeptides, lipopeptides and lipoglycopeptides

The exact mechanism of glycopeptide resistance among CoNS is still unknown. There are three phenomena leading to glycopeptide therapy failure that have been postulated in staphylococci (mostly in SA):

1.-The development of vancomycin-intermediate SA (VISA) isolates and the recognized precursor subpopulations named hetero-resistant VISA (h-VISA), identified in 1996 (Hiramatsu, Hanaki, et al. 1997). Since, these isolates can also be resistant to teicoplanin, the term glycopeptide-intermediate SA (GISA and h-GISA) is also used. Their complex resistance mechanisms include cell wall alterations (reorganization and thickening) and reduced autolytic activity (Cui et al. 2006; Boyle-Vavra et al. 2001; Howden et al. 2010). Cell wall thickening has also been reported for CoNS (*S. epidermidis* and *S. haemolyticus*)(D Sanyal & Greenwood 1993; Biavasco et al. 2000). Glycopeptide-resistant CoNS may have an excess of glycopeptide binding sites due to the overproduction of peptidoglycan in the bacteria cell wall (Biavasco et al. 2000). Thus, the basic mechanisms leading to glycopeptide resistance, although highly heterogeneous, appear to be similar between CoNS and SA.

2.-The development of VRSA isolates. The first vancomycin-resistant SA (also glycopeptide-resistant strains) VRSA and GRSA, containing the gene *van*A was isolated in 2002 in US (Centres for Disease Control and Prevention (CDC) 2002). Although the acquired vancomycin-resistance determinants (*van*A, *van*B, *van*D, *van*E, *van*F and *van*G) had been reported from vancomycin-resistant enterococci (VRE), this was the first time that these determinants were isolated in SA(Woodford 2001). The presence of *van*A in this VRSA was suggested to have been acquired through exchange of genetic material from the VRE also isolated from the same

patient. Conjugative transfer of the *van*A gene from enterococci to SA had been demonstrated previously *in vitro* and *in vivo* (Noble et al. 1992). In Europe, the first case of infection with VRSA occurred in Portugal in 2013 in 74-year old woman who underwent amputation of two gangrenous toes-isolated from the pus of the toe amputation wound. The vancomycin and teicoplanin MICs were >256 and 24 mg/L, respectively. The strain was sequence type ST105, SCCmec type II and harboured the *mec*A and *van*A genes. The concomitant isolation of VRE (*Enterococcus faecalis*) suggested the possible source of the *van*A gene for the acquisition of the vancomycin resistance, similarly to the cases reported previously in the US (Melo-Cristino et al. 2013).

In CoNS, the implications of a recent report on highly vancomycin resistant strains (MIC≥ 256 mg/L tested by Etest) recovered from the saliva of migratory birds in Kansas and harbouring *van* genes is unknown (Ishihara et al. 2013). A recent observation of 3 *S. epidermidis* isolates containing the *van*A and *van*B1 genes within a collection of 30 isolates from ICU patients in Kampala (Uganda) warrants also further evaluation (Okee et al. 2012).

3.-The so-called phenomenon "Vancomycin MIC creep". This event describes a population drift in clinical SA isolates towards reduced vancomycin susceptibility, but with MICs values that are still below the susceptibility breakpoint (Steinkraus et al. 2007; Wang et al. 2006). However, it is still a matter of debate as there are other several studies that have not shown any changes or reductions on the vancomycin MICs over time (Holmes & Jorgensen 2008; Musta et al. 2009). To date, there have not been reports of vancomycin MIC creep in CoNS, although there is a high and increasing prevalence of heterogeneous glycopeptide intermediate resistance (hGIS)(Ahlstrand et al. 2011).

The following table summarizes the reports on glycopeptide (teicoplanin and vancomycin) resistance or reduced susceptibility among staphylococci recovered from patients with descriptions on their likely underlying mechanism(s).

Study	Study Characteristics	Isolates	MIC_{90} /Range (method)	% Resistance (total number)	Mechanism	Notes
(Schwalbe 1986)	Case report of a patient with diabetes and end-stage renal disease with repeated episodes of peritonitis caused by CoNS (with CAPD)	*S.haemolyticus* (2 or 3 different isolates-out of 8 recovered-according to biochemical patterns) from peritoneal fluid	Teicoplanin: 8-64 mg/L Vancomycin: 2-8 mg/L (microdilution procedure with modifications) (NCCLSI)	NR	-The authors suggest that CoNS have the ability to acquire vancomycin resistance. -Mechanism is not clear.	-Stepwise increase in vancomycin resistance from MICs of 2 to 8 mg/L over 88 days of vancomycin therapy. -Teicoplanin was ineffective in a uniform way and no stepwise resistance was seen.
(Goldstein et al. 1990)	Investigation of the activities of teicoplanin and vancomycin against CoNS during Nov 1988-April 1989	CoNS (n= 362 clinical isolates) *S.epidermidis* (74%) *S.haemolyticus* (19%)	Teicoplanin:0.5-32 mg/L Vancomycin: 0.5-8 mg/L (Agar dilution method, CLSI)	Teicoplanin: R: 1.7% and 23.2% intermediate R Vancomycin: < 0.3% R Interpretative criteria NCCLSI for vancomycin breakpoints (applied to teicoplanin in this study): S ≤4, I: 8-16 and R ≥32 mg/L)	NR	Most of the teicoplanin resistant strains were isolated from patients with no previous exposure to teicoplanin or vancomycin
(Sanyal et al. 1993a)	Study of the *in vitro* characteristics of glycopeptide-resistant strains through passage in glycopeptide-containing and free broth	3 isolates of *S.epidermidis* isolated from the peritoneal fluid of two patients on CAPD from the same renal unit. Patients were previously exposed to vancomycin.	Teicoplanin: 32 mg/L Vancomycin: 4-32 mg/L (Broth dilution method)	Teicoplanin: R 100% Vancomycin: R 33.3%, I: 33.3%, S: 33.3% Interpretative criteria NCCLSI for vancomycin breakpoints (applied to teicoplanin in this study): S ≤4, I: 8-16 and R ≥32 mg/L)	Constitutive resistance (not inducible) and not increased by passage in the presence of vancomycin or teicoplanin. Resistance phenotype stable (after 25 sequential sub-cultures)	- There was a loss of antibiotic effect when cultures were exposed to sub-MIC concentrations and grew to stationary phase, more marked for teicoplanin. The authors suggest this could be due to an increased capacity of the cell wall of resistant strains to bind to glycopeptides (at sites other than D-

						alanyl-D-alanine target site).
(Hiramatsu, Aritaka, et al. 1997)	The prevalence of VRSA (Mu50) and Mu3-like strains (MIC 3 mg/L) was investigated in 203 hospitals in Japan	-1149 MRSA isolates from Japanese hospitals	-h-VRSA vancomycin MIC range (1-4 mg/L) by NCCLS method. MICs were determined with BHI agar plates and 1mg/L vancomycin increments (1-10 mg/L)-concentration able to inhibit $5x10^5$ CFU/mL after 16 h incubation- and compared with micro-broth dilution by NCCLS method.	-Only 1 strain/1149 (N20) in addition to Mu50 had a vancomycin MIC of 4 mg/L (NCCLS method). This strain produced subclones with a vancomycin MIC of 8mg/L at a high frequency of 10^{-4} -A 2.96% (34/1149) was h-VRSA, able to grow sub-populations in the presence of vancomcyin 8 mg/L	-The authors suggest that vancomycin resistance of VRSA appears to be achievable without the acquisition of extrinsic genes, by two-step drug selection of originally vancomcyin susceptible MRSA of clonotype II-A in Japan.	-PFGE pattern similar in Mu50 and the h-VRSA strains with clonotype II-A (about a 70% of all MRSA strains in Japan in the 90´s), characterized by the production of coagulase type-2 and TSST-toxin-1.
(Sieradzki et al. 1998)	Single-hospital study during a 5-month period (between 1995-1996) to investigate decreased susceptibilities of teicoplanin and vancomycin against CoNS	41 MR-CoNS -24 different patterns in 28 isolates tested for clonality. -Most of the isolates were suspected to be involved with infection	Teicoplanin: 4-16 mg/L after 48 h 8-32 mg/L after 72h Vancomycin:2-4 mg/L (Standard broth microdilution method-CLSI)	Teicoplanin: 68.3% low-level R (28/41) Vancomycin:0% R but heterogeneous subpopulations could grow on cultures containing 6-12 mg/L of vancomycin.	- Sub-MIC teicoplanin concentrations produced**:** inhibition of autolysis and formation of cellular aggregates, which disintegrates in the stationary phase to individual bacteria, with removal of antibiotic, similarly	-*S. epidermidis (n=5)* and *S.haemolyticus (n=1)* (ATCC strains, n=2 from the pre-glycopeptide era) were also used for PAPs experiments, showing all homogeneous susceptibility to vancomycin but heterogeneous reduced susceptibility to teicoplanin, suggesting species intrinsic

					to what has been previously described in vancomycin R MRSA.	resistance phenotypes in the latter.
(Tacconelli et al. 2001)	One year prospective case-control study (1:3) to assess the incidence, risk factor and genotypic patterns of glycopeptide-resistant CoNS bacteraemia	535 CoNS isolates from BSI: *S. epidermidis* (70%), *S.hominis* (12%), *S.haemolyticus* (9%), *S.capitis* (9%), *S.warneri* (2%), other CoNS (1%)	No MIC range reported. Broth microdilution (NCCLSI) and Etest Teicoplanin resistance defined as an MIC≥ 18 mg/L and for vancomycin MIC≥ 8 mg/L	Teicoplanin R: 3.5% (19/535) R Both teicoplanin and vancomycin R: 0.19 %(1/535) Glycopeptide R: 3.7% (20/535); MR-CoNS: 69.5%	NR	-Different patterns with a high degree of polymorphism (ribotyping and computer analysis of fingerprinting) -Previous exposure to β-lactams and glycopeptides, multiple hospitalization and concomitant pneumonia were the risk factors of glycopeptide resistance CoNS BSI.
(Arias et al. 2003)	Multi-centre prospective surveillance of antimicrobial resistance in enterococci and staphylococci in Colombian hospitals (2001-2002)	Isolates: -CoNS (177) -SA (296) -MRSA (155) -MSSA (141) from clinical samples	Teicoplanin/Vancomycin: -0.12-4 / 0.12-4 mg/L -0.06-4/ 0.25-4 mg/L -0.06-4/2-6 mg/L -0.25-4/0.25-4 mg/L (Agar dilution method: NCCLSI)	Both teicoplanin and vancomycin: -0% -0% -0% -0% -Oxacillin-R: 73%	NA	-All isolates were investigated with PCR multiplex assay for the detection of the *mec*A gene. -All MRSA isolates were screened for VISA -In enterococci, resistance to glycopeptides was 9.7 % (presence of *van*A:58.3% and

						*van*B: 41.7% genes)
(Cui et al. 2003)	Investigation of cell wall thickness in clinical VRSA strains through daily passage in drug-free and vancomycin containing medium.	-16 strains of clinical VRSA from 7 different countries -48 strains underwent cell wall thickness and MIC determination	-Initial MIC range of parental strains: -Vancomycin: 1-10 mg/L -Teicoplanin: 2-29 mg/L -Imipenem: 1-64 mg/L -Oxacillin: 0.25-1024 mg/L (MIC test done with BHI agar and 1 mg/L of drug increments, except for oxacillin, where they used conventional 2-fold dilution)	-From subcolonies of passage-derived strains grown on plates containing 4 mg/L vancomycin: -Vancomicn MIC range 5-9 mg/L with a frequency of $4.25x10^{-6}$ to $1.64x10^{-3}$ -Teicoplanin MIC: 6-32 mg/L -Imipenem: 1-64 mg/L -Oxacillin: 1-512 mg/L *(Association between the conversion of VSSA to h-VRSA with hetero-to homoconversion of MR-caused by β-lactam selection)	-Cell wall thickness may be a phenotypic determinant for vancomycin resistance in SA. -The authors also suggest that the use of β-lactams in MRSA infection might be a risk factor for the emergence of h-VRSA, but the mechanism remains unclear*	-All of the VRSA strains had thickened cell walls, which became thinner with the loss of vancomycin resistance and decreased MICs after drug-free passages but became thick again (all except one of the parent strain passage-derived) after 1 step of vancomycin passage.
(Trueba et al. 2006)	Retrospective 4-year (2000-2004) study of all staphylococci collected in the laboratory from clinical samples	N=2476 staphylococci -CoNS (1039): -*S.epidermidis* (60.8%), *S.haemolyticus* (9.9%), -*S.hominis* (13.2%), -other CoNS (16.1%) -SA(1437)	AST method (French Society of Microbiology): 10 µl of $6x10^8$ CFU/mL on MH agar plates with 5 mg/L teicoplanin. Readings made after 24h at 37ºC. Teicoplanin resistance MIC > 8mg/L.	2000 7.2% 17.9% 9.5% 4% 2.8%	2001 17.2% 25% 7.4% 10.3% 1.3%	2002 35.2% 55.5% 21% 14.6% 0.9%	2003 46.1% 31.2% 20.9% 5.9% 0.9%	2004 30.4% 35.7% 14.8% 8.7% 1.2%	NR -Co-resistance with ciprofloxacin occurred in 60% of the *S. epidermidis* strains	-The authors do not relate the marked rise of teicoplanin resistance among *S. epidermidis* isolates with spread of any predominant clones or with teicoplanin consumption.
(Kratzer et al. 2007)	In vitro study of the activity of daptomycin and	N= 105 CoNS strains from 76 bone marrow	Vancomycin MIC_{90}= 2 mg/; Daptomycin: 0.25 mg/L; Tigecycline: 0.5	-All strains were susceptible to daptomycin, tigecycline and fosfomycin.					NR	

	tigecycline compared to vancomcyin and fosfomycin (strains collected from 2000-2006)	transplant patients with BSI (n=102 *S.epidermidis*, n=3 *S.haemolyticus*)	mg/L; Fosfomycin: >250 mg/L (CLSI method)			
(Kuti et al. 2008)	PD study of glycopeptide exposures against clinical isolates of staphylococci collected from hospitals in Brazil (2003-2005)	Isolates: -SA (119) -MSSA (79) -MRSA (40) -CoNS (82) -MS-CoNS (8) -MR-CoNS (74)	Teicoplanin/Vancomycin 0.25-4/1-4 mg/L 0.25-4/1-2 mg/L 0.5-4/ 1-4 mg/L 0.5-64/1-4 mg/L 1-8/1-2 mg/L 0.5-64/1-4 mg/L (Etest and interpreted according to CLSI: R≥32 mg/L for CoNS teicoplanin and vancomycin and I: 4-8, R≥ 16 for SA for vancomycin)	Teicoplanin/Vancomycin R: -0%/ I:0.8% -0%/ 0% -0%/ I: 2.5% -3.7%/0% -0%/0% -4.1%/0% *90.2% MR-CoNS	NR	-Linezolid showed to have a greater probability of attaing the PD target (AUC/MIC > 82.9) than teicoplanin and vancomycin did (AUC/MIC > 345) against staphylococci.
(Hope et al. 2008)	Multi-centre 5-year study involving 25 hospitals in UK and Ireland, each collecting up to 10 consecutive isolates of both SA and CoNS per year	-SA (1448) -MSSA(840) -MRSA (608) -CoNS (1214) -MS-CoNS (401) -MR-CoNS (813)	Teicoplanin/Vancomycin: -0.5-8/0.5-4 mg/L -0.5-16/0.5-4 mg/L -0.12-32/0.5-4 mg/L -0.12-32/0.5-8 mg/L	Teicoplanin: -0% R -0.2% R -1.5% R (7.6% Intermediate R) -3.1% R (23.4% Intermediate R), only one strain with vancomycin intermediate resistance, MIC 8 mg/L	NR -Thirty-four CoNS isolates from 2006 (17%) had the *mup*A (not sought before) gene and only two isolates lacked the *mec*A gene.	-The prevalence of MR-CoNS (about a 70%) was strongly associated with multi-resistance, particularly with ciprofloxacin, clindamycin, rifampicin, teicoplanin and trimethoprim.
(Natoli 2009)	Hospital microbiological records reviewed to identify reduced susceptibility to glycopeptides	-CoNS (1609) isolates from patients with BSI in ICU and haematological transplant	Teicoplanin MIC range: - 2-16 mg/L (in transplant ward isolates) - 4-32 mg/L (ICU wards) Vancomycin MIC range: -2-4 mg/L in both wards	-5.4% of CoNS (87/1609) showed reduced susceptibility to glycopeptides and of those, -Only 5.7% of the total (92/1609) and 14.9% of those with reduced glycopeptide susceptibility (13/87)	NR -Strains were induced to express glycopeptide resistance by culture on	-Infections caused by CoNS with reduced glycopeptide susceptibility, represented 11% and 20% of all CoNS-

		wards	(EUCAST interpretative criteria, automated system and Etest)	considered to be associated with infection (11 *S.epidermidis* and 2 *S.haemolyticus*) -53.8 % of those associated with infection and reduced susceptibility, (7/13) showed a teicoplanin MIC ≥ 8 mg/L and all (13/13) vancomycin MIC≥ 2 mg/L	vancomycin agar screen plates (CLSI and CDC guidelines) with 6 mg/L of vancomycin.	related BSI in haematology transplant ward and ICU, respectively in this study
(Ahlstrand et al. 2011)	Retrospective single-center study where all positive blood cultures with CoNS were re-evaluated during 3 decades for the presence of reduced susceptibility to glycopeptides	Bloodstream isolates of S.epidermidis (387) and S.haemolitycus (19) from patients with haematological malignancies	NR for teicoplanin/ For vancomycin median MIC 2 mg/L (Standard Etest, macromethod Etest and GRD Etest)	-31-45% hGIS (macromethod test) -53-67% (GRD Etest)	NR	- This study shown non-long term glycopeptide MIC creep as per standard Etest.
(Karlowsky et al. 2011)	In vitro activity of dalbavancin and telavancin against staphylococci and streptococci from patients in Canadian hospitals (2007-2009)	Isolates -MSSA (1980) -MRSA (631) -MSSE (202) -MRSE (34)	Vancomycin: -MIC_{90} 1 (0.25-2) mg/L -MIC_{90} 1 (0.5-4) mg/L -MIC_{90} 2 (0.12-4) mg/L -MIC_{90} 2 (1-2) mg/L CLSI method and interpretative criteria 2016.	Vancomycin R: 0% 0% 0% 0%	NA	The rank order of potency (based on MIC_{90} of glycopeptides against both SA and *S.epidermidis* was dalbavancin (0.06 mg/L) >telavancin (0.5 mg/L) > vancomycin (1-2 mg/L)
(Guzek et al. 2013)	Collection of staphylococci and enterococci strains from patients treated in the ICU,	Isolates: -CoNS (37) -MSSA (89) -MRSA(24) -*E.faecium* (11)	Teicoplanin/ Vancomycin 0.094-8/0.75-2 mg/L 0.19-2/0.5-2 mg/L 0.38-1.5/0.38-1.5 mg/L 0.094-0.5/0.38-1 mg/L	-Teicoplanin -16.2% (6/37) -0% -0% -0%	NR	-In this study, teicoplanin exhibited greater clinical efficacy against enterococcal

	surgical and Muskuloskeletal infection units of the Military Institute of Medicine (Warsow, Polonia)	-*E.faecalis* (39)	0.047-0.75/0.38-3 mg/L Etest and EUCAST interpretative criteria (teicoplanin R in CoNS> 4mg/L, in SA >2 mg/L)	-0% -All isolates were susceptible to vancomcyin		infections, efficacy of vancomcyin nd teicoplanin against MRSA was comparable and vancomcyin displayed mora favourable MICs than teicoplanin against CoNS.
(Sader et al. 2013)	Study of the *in vitro* activity of ceftaroline against staphylococci from US hospitals with decreased susceptibility to linezolid, daptomycin or vancomycin (2008-2011)	-Isolates: -SA (19350) -MRSA (9875) *A 51% MR-SA -CoNS (3270) -MR-CoNS (2268) *A 69.4% MR-CoNS	Vancomycin MIC_{90} -1 mg/L -1 mg/L -2 mg/L -2 mg/L	Resistance/decreased susceptibility among SA: -Linezolid: 0.07% (14 strains). MIC≥8 mg/L -Daptomycin: 0.09% (18). MIC≥2 mg/L -Vancomycin: 1.9 % (369) MIC≥2 mg/L Resistance/decreased susceptibility among CoNS: -Linezolid: 1.6% (51 strains), MIC≥8 mg/L -Daptomycin:0.12% (4), MIC≥2 mg/L -Vancomycin: NR	NR	Ceftaroline showed a potent in vitro activity against SA and MRSA (MIC_{90} 1mg/L) and against CoNS isolates (MIC_{90} 0.5 mg/L), including MR-CoNS (MIC_{90} 0.5 mg/L), linezolid-resistant (MIC_{90} 0.5 mg/L) and daptomycin non-susceptible strains (MIC 0.03-0.12 mg/L)

ATCC: American Type Culture Collection; BHI: brain heart infusion; BSI: Bloodstream infection; CAPD: Continuous Ambulatory Peritoneal Dialysis; CFU: Colony Forming Units; CDC: Centers for Disease Control; NCCLSI: former CLSI: Clinical and Laboratory Standards Institute; GRD: Glycopeptide resistance detection; ICU: Intensive Care Unit; MR: Methicillin-resistance; MS: Methicillin-susceptible; NR: Non-reported; NA: Non-applicable; R: resistance; S: Susceptible; I: Intermediate; PAP: population analysis profile; PCR: polymerase chain reaction; PFGE: Pulsed-field gel electrophoresis; TSST: Toxic shock syndrome toxin.

Table 1.1- Glycopeptide (vancomcyin and teicoplanin) resistance or reduced susceptibility reports among staphylococci.

Daptomycin is a bactericidal lipopeptide that also targets the cell membrane of Gram-positive bacteria. Several mechanisms leading to resistance have been studied, mostly in SA, however, they have not been completely clarified. For instance, increased cell wall teichoic acid production and their D-alanylation with progressive cell wall thickening, may contribute to daptomycin resistance (Bertsche et al. 2013). In addition, several single nucleotide polymorphisms in various gene loci (e.g. the multipeptide resistance factor gene, *mpr*F) have been identified (Friedman et al. 2006). So far, daptomycin has exhibited potent activity against CoNS as it was shown in a multi-centre surveillance study with 42 countries involved, with more than 22.000 clinical isolates investigated over 9 years (2002-2010). In this study, daptomycin inhibited 99.8% of CoNS at ≤ 1mg/L (susceptibility breakpoint) and was up to 16-fold more active than vancomycin (Sader & Jones 2012).

The lipoglycopeptides, dalbavancin, oritavancin and telavancin possess potent *in vitro* activity against staphylococci with lower MIC values than those of vancomycin and teicoplanin (Zhanel et al. 2010). To date, there has not been single-step, high level (frequencies < 10^{-10}) dalbavancin, oritavancin or telavancin resistance. After 24 passages of SA and *S. haemolitycus* at sub-MIC concentrations, dalbavancin MICs increased 2-fold for SA and 4-fold for *S. haemolyticus,* however the causes have not been fully elucidated (Kim et al. 2007). For oritavancin, despite the *in vitro* demonstration of moderate-level resistance (MIC ≤16 mg/L) among enterococci harbouring *van*A and *van*B genes (Arthur et al. 1999); there have not been cases of resistance among clinically isolated pathogens. *In vitro*, telavancin has shown a low incidence of spontaneous resistance among staphylococci and enterococci (Kosowska-Shick et al. 2009).

Resistance to oxazolidinones

For linezolid, three mechanisms have been elucidated that produce resistance in staphylococci: 1) accumulation of single point mutations in the 23S rRNA (domain V)-binding site in at least two copies of the rRNA gene operons; 2) more infrequently, mutations in the *rpl*C and *rpl*D genes in the peptide translocation centre of the ribosome, encoding the 50S ribosomal proteins L3 and L4, respectively and 3) acquisition of the plasmid-borne ribosomal methyl-transferase gene, *cfr* (Kloss et al. 1999)(Pillai et al. 2002; Locke et al. 2009; Toh et al. 2007). The latter, *cfr*-mediated resistance is of serious concern due to its possible high horizontal transfer capability. For instance, a multi-centre outbreak of a *cfr*-containing linezolid resistance *S.epidermidis* has already been reported (Bonilla et al. 2010). Furthermore, an isolate of *S.epidermidis* was reported in Spain, after an outbreak of *cfr*-mediated linezolid resistant SA in the ICU, containing a concurrence of all three described mechanisms leading to linezolid resistance (Baos et al. 2013). Nonetheless, to date, linezolid resistance is rare but concerns are growing.

Resistance to tetracyclines and glycylcyclines

The resistance to tetracyclines among staphylococci and other range of bacteria remains on the acquisition of mobile genes (*tet* and *otr*), which leads to ribosomal protection via the dissociation of the drugs from their ribosomal binding sites, and also to drug efflux via the active transportation of tetracyclines outside the bacterial cell (Connell et al. 2003; Butaye et al. 2003). There have been reports of tetracycline-resistant CoNS isolates, being higher the prevalence in MR-CoNS (18.6%) than in methicillin-susceptible ones (7.6%) (Flamm et al. 2015).

Tigecycline (minocycline derivative compound) represents a new class of glycylcyclines with bacteriostatic broad-spectrum activity that overcomes the development of resistance of classical cyclines, as it is not a substrate for tetracycline efflux pumps (Chopra et al. 2002). For staphylococci, the mechanism(s) of tigecycline resistance remains unknown, however, *in vitro* decreased susceptibility among MRSA has been reported through a novel family of efflux pumps (*mep*A) (McAleese et al. 2005). A year after the introduction of tigecycline, a surveillance multi-centre study carried out in Germany in 2007, reported on *S. epidermidis* (142 isolates) and SA (308 isolates) susceptibility to tigecycline with MIC_{90} in the range ≤ 0.125-0.5 mg/L. Only three isolates of *S.haemolitycus* (4.5% of *S.haemolitycus*) exhibited an MIC over the breakpoint (> 0.5 mg/L) (Kresken et al. 2009).

Resistance to fusidic acid, fosfomycin and rifampin

These agents, although considered "old antibiotics" are increasingly being considered because of its still potent activity against Gram-positive (fusidic acid) or against both Gram-positive and Gram-negative bacteria (fosfomycin and rifampicin). However, they develop rapid resistance if used in monotherapy, therefore they are mainly used in combination, despite the lack of PK/PD evidence to support these strategies and basically being based on clinical experience.

For fusidic acid, there exist spontaneous mutations in the *fus*A gene that dispose to an altered ribosomal translocase, the elongation factor G, which is the drug target for protein synthesis inhibition. A *fus*B mechanism has also been described for staphylococci that can be chromosomal or plasmid-mediated, and that encodes an inducible elongation factor G-protecting protein. Particularly, in SA and *S.saphrophyticus*, *fus*C and *fus*D homologs, respectively, have also been reported (O'Neill et al. 2007). In a multi-centre study in 13 European countries with a

collection of 3.134 staphylococci isolates, fusidic acid resistance (MIC≥ 2 mg/L-EUCAST) ranged between a 10.7% in SA strains (288/2700) to a 37.5% in CoNS isolates (155/434) (Castanheira, Watters, Mendes, et al. 2010). On a more global basis, a study including the US, Canada and Australia, among clinical staphylococci (n=4167 SA and n=790 CoNS), only a 1.7% overall displayed fusidic acid resistance. This was higher among CoNS ranging from 7.2% in US to 20% in Canada (Castanheira, Watters, Bell, et al. 2010).

Resistance to fosfomycin can be mediated by either chromosomal or plasmid-borne mechanisms. Relatively little is known about chromosomally mediated resistance in Gram-positive bacteria (defects in the transport system that takes up fosfomycin-*glp*T or *uhp*T-)(Młynarczyk et al. 1985). In plasmid-mediated, *fos*A gene encodes a glutathione S-transferase that inactivates fosfomycin (Llaneza et al. 1985). In staphylococci, a homolog *fos*B has also been reported (Etienne et al. 1989). A Greek study from 2008 including 1846 Gram-positive bacterial isolates (disk diffusion method for susceptibility test), reported a 22.5% of CoNS (216/961) displaying *in vitro* resistance to fosfomycin. However, in this study only a 0.7 % of SA (3/419) including MRSA exhibited fosfomycin resistance (Falagas et al. 2009).

Rifampicin resistance in staphylococci is most frequently due, but not only, to point mutations in the regions of the *rpo*B gene, which encodes the β-subunit of the bacterial RNA polymerase and leading to aminoacid substitutions at or near the drug-binding site (Tupin et al. 2010). In a Swedish study investigating the susceptibility among *S.epidermidis* isolated form prosthetic joint infections, approximately a 39% of isolates were resistant to rifampicin (MIC> 32 mg/L) and about a 15% of CoNS tested resistant in a multi-centre study in Colombia (Hellmark et al. 2009; Arias et al. 2003).

Resistance to mupirocin

Reports on the increasing prevalence of high-level mupirocin resistance in CoNS reaching a 61% are of serious concern as this topical antibiotic is widely used in nasally colonized with MRSA patients. In addition, this resistance is mediated by plasmids carrying the gene *mup*A, which can be transferred to SA. These plasmids are also associated with genes encoding resistance to multiple antibiotics (Bathoorn et al. 2012; Lepainteur et al. 2013). Resistance to mupirocin in SA is also reported to be widespread in children. Routine mupirocin testing might be essential before MRSA decolonization strategies or the topical treatment of skin infections (Antonov et al. 2015).

Resistance to biocides and antiseptics

Biocides and antiseptics conform an important part of the infection control armamentarium, especially in the ICUs. In a recent study from a French neonatal ICU, a 41.2% of CoNS isolates recovered from CLABSIs in preterm neonates, presented reduced susceptibility to at least one used antiseptic (12% to chlorexidine; 24% to benzoalkanium and 33% to acriflavine), which is of concern as several resistance determinants are located on plasmids and transposons with resistance genes encoding antibiotic resistance (Lepainteur et al. 2013).

1.4- PHARMACOLOGICAL APPROACHES TO OPTIMISE ANTIMICROBIAL THERAPY IN CHILDREN

In the paediatric population, there is often suboptimal drug dosage information that does not take into account the developmental changes that influence drug disposition across childhood. Simplified dosing approaches and extrapolation from adult studies have been proved to be inadequate since they are based on assumptions around the idea that human growth is a linear process (Kearns et al. 2003). There is surprisingly very little pharmacological data that supports regimens that are widely used in children and neonates. In addition, many doses in existing formularies, including the British National Formulary for Children, are dated from more than two decades ago and lacking evidence (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015b). As a consequence, inadequate or sub-therapeutic dosing could lead to increased preventable drug-related toxicity or ineffective treatment with the subsequent potential for developing antimicrobial resistance.

Neonates and children have important differences when compared to adults in terms of drug absorption, disposition, metabolism and elimination (Kearns et al. 2003). There are also significant differences in the pathophysiology of underlying diseases and in the epidemiology and resistance patterns of organisms infecting children and neonates (Biedenbach et al. 2004). The optimal definition of dosage regimens in children requires and in-depth understanding and targeted study of these differences. This major research gap has been addressed with the introduction of the European Union Paediatric Regulation in 2006 to ensure that any medicinal product for use in children undergoes extensive studies before use in the target population,

but also with global research initiatives in Paediatrics (GRIP), a European Union-funded research network that promote the developing of our understanding of paediatric clinical pharmacology (The European Parliament and The Council of the European Union 2006).

Therefore, there is an urgent need to update current dosage regimens with new high-quality evidence, based on pre-clinical and clinical data derived from target populations, that accounts for its specific population developmental and disease-specific variability as well as for the changing patterns of infection and resistance.

1.4.1- Understanding the differences in the clinical pharmacology of antimicrobials in children: developmental pharmacology

Dosing equations largely used in the past (e.g Young´s formula) has been replaced by normalization of the drug dose to either body weight or body-surface area. This is in recognition that human growth is not a linear process across childhood, and that many age-related changes occur in body composition and organ functionality, which affect the drug´s pharmacokinetic behaviour and thus, its pharmacodynamics. Nonetheless, this approach might be adequate for initiating therapy, but it is certainly insufficient during maintenance dosing or long-term treatment, where regimens should be individualized according to the different PK, PD or both of patients.

For instance, the relatively larger extracellular and total-body water spaces in neonates and young infants as compared with adults, combined with a higher ratio of water to lipid in the adipose tissue, results in lower plasma drug concentrations in these compartments for hydrophilic drugs. In contrast, the influence of age on the apparent volume of distribution is not as marked for lipophilic drugs, which are

primarily distributed in the tissues (Singh et al. 2001). Furthermore, age-related changes in the composition and amount of plasma proteins (e.g. albumin) can also affect the distribution of highly protein bound drugs (Grandison & Boudinot 2000). A decrease in the amount of total plasma proteins in the neonate, for example, increases the free fraction of the drug and thus, determines the exposure of the active moiety. In addition, during the neonatal period, an increase in endogenous substances, such as bilirubin, and the presence of foetal albumin, are able to displace a drug from albumin binding sites, contributing also to the higher availability of the free actions of highly protein bound drugs (e.g ceftriaxone, teicoplanin, etc)(Sethi et al. 2016).

In terms of drug metabolism, a consistent observation in clinical studies is an age-dependent increase in plasma clearance of drugs metabolized by the liver in children < 10 years old as compared with adults. Therefore, in these cases, younger children necessitate relatively higher weight-based dosing of these drugs (Singh et al. 2001). The activity of many cytochrome P-450 (CYP) isoforms and a single glucuronosyl-transferase (UGT) isoform is markedly reduced during the first two months of life. Distinctive patterns of isoform-specific developmental changes in the biotransformation of drugs are apparent for phase I (oxidation) and Phase II (conjugation) drug-metabolizing enzymes. The acquisition of adult activity over time is enzyme and isoform-specific (Kearns et al. 2003). *Literature review on CYP ontogeny has been deliberately reduced as the drugs involved in this thesis (vancomycin and teicoplanin) are mostly (90 and 98% respectively) renally cleared* (De Hoog et al. 2004)(A. P. R. Wilson 2000).

In relation to the renal elimination of drugs, is now well described that nephrogenesis begins at 5 weeks gestation and is complete by 36 weeks gestation,

followed by postnatal changes in organ blood flow. The GFR increases rapidly during the first two weeks of postnatal life and then rises progressively until adult values, reached at 8 to 12 months of age (Rhodin 2009). Similarly, tubular secretion is immature at birth and reaches adult capacity during the first year of life.

Collectively, developmental changes in renal function can alter considerably the plasma clearance of drugs that are extensively renally eliminated. Therefore, knowledge of these changes is fundamental in the adequate age-selection of optimal dosing (e.g ceftazidime)(van den Anker et al. 1995) as well as in dose intervals (e.g gentamicin)(Brion et al. 1991). Concomitant medication may also alter the normal pattern of renal maturation in neonates (van den Anker et al. 1994).

Failure to account for all these differences can expose neonates and young infants, particularly, to potentially toxic levels of drugs.

Allometric scaling of clearance in paediatric pharmacology and PK modelling

Body size is an important determinant of drug exposure in children. Children have higher dose requirements on a $mg*kg^{-1}$ basis compared to adults due to the fact the clearance scales to body surface area by raising weight to a power that ranges around 0.63 (glomerular filtration) to 0.78 (hepatic clearance)(Rhodin 2009; Johnson et al. 2005). Allometry is the study of the relationship between body size and physiology. Allometric scaling for size with a fixed exponent of 0.75 is widely accepted to predict or describe clearance of drugs in children adequately, whilst adding biological information on the effect of body size without adding an extra parameter to be estimated. Conclusively, smaller people will have shorter half-lives due to their proportionately higher clearance and thus, elimination constant (Standing 2016).

However, this approach does not perform as well in neonates and young infants due to the rapid physiological and maturation changes in drug elimination that occur early in life. Therefore, a method for scaling for both size and maturation is needed. The use of both allometric scaling of clearance to size and the use of a sigmoid maturation function driven by PMA has been proposed, as follows:

$$Cl = Clt * \left(\frac{wt}{70}\right)^{0.75} * \frac{PMA^{Hill}}{(PMA50^{Hill} + PMA^{Hill})}$$

Where Cl=clearance (L/h), Clt= clearance of a typical 70 kg adult (L/h), wt= body weight (kg), PMA= post-menstrual age (weeks) and Hill= the Hill coefficient or shape parameter.

Recently, a systematic comparison of models for scaling clearance in children showed that several published modelling approaches gave similar fits to the data and no model out-performed the standard model proposed above. This highlighted the added benefit of standardising scaling using a single method to facilitate paediatric population PK modelling knowledge between studies (Germovsek et al. 2016).

Finally, the apparent volume of distribution should scale linearly with body weight since blood volume is proportional to weight, especially for highly protein-bound drugs, such as teicoplanin (Standing 2016).

1.4.2- The role of PK/PD determination and analysis in optimising antimicrobial therapy

Modern antimicrobial pharmacology postulates that the shape of the antimicrobial concentration-time curve relative to the microbial susceptibility- the latter represented by the pathogen´s minimum inhibitory concentration (MIC)-, is an important determinant of drug effectiveness. Three summary PK parameters are used

to capture this information: 1) the time of the dosing interval that the non-protein bound, or free fraction (*f)* of the drug, is able to maintain a concentration above a threshold, the MIC, (% *f*T>MIC). This time-dependence index is suggested to be the best descriptor of the activity for some antimicrobials (e.g. β-lactam antibiotics); 2) the peak concentration achieved (fC_{max}/MIC), which better describes the concentration-dependent activity for other antimicrobials (e.g aminoglycosides); and 3) the area under the concentration-time curve (*f*AUC/MIC), which may be more important in predicting therapeutic success in drugs with both concentration and time-dependence activity, such as in quinolones and glycopeptides, for instance (Mouton et al. 2005; Roberts et al. 2014).

The rationale behind which PK/PD index best correlates with efficacy depends on several factors such as the mechanism of action, the microbial kill kinetics, the degree of protein binding and the degree of tissue distribution. In general, antimicrobials with a mechanism of action of inhibition of either protein or bacterial synthesis, display concentration-dependent killing and correlate well with either *f*AUC/MIC or fC_{max}/MIC (e.g aminoglycosides). On the other hand, antimicrobial agents that act on the cell wall synthesis display mainly time-dependent activity and correlate with % *f*T>MIC (e.g β-lactams). However, a major exception is with antimicrobials that display a prolonged post-antibiotic effect (PAE) and correlate better with *f*AUC/MIC despite the mechanism of action (Barbour et al. 2010).

Knowledge of these PK/PD indices are used in the design of dosing strategies that aim to achieve PD targets that are associated with optimal antimicrobial effects for any specific drug/organism combination. Clinicians may also use this information to inform their choice in the way a drug is administered as well as in dose

modifications or adjustments, which may be required in different clinical scenarios, populations and individual patients.

The introduction and development of *in vitro* and *in vivo* infection models over the past three decades, have allowed accurate study of PK/PD relationships and definition of optimal PK/PD targets for "old" and new drugs that are associated with maximal effect and suppression of resistance in patients. Importantly, recent clinical analysis have described, in the majority of cases, similar PK/PD optimal targets in patients to those that have been observed in pre-clinical studies, thus confirming the utmost relevance of the latter studies as the first step in getting the dosing regimens right the first time that are studied in humans (Roberts et al. 2014). In summary, the role of PK/PD studies is becoming increasingly recognized in drug development, in revisiting and building the evidence behind dosing recommendations used in well-established antibiotics in adults and especially in paediatrics, as well as in the understanding and tackling of the antimicrobial resistance emergence problem.

In vitro PK/PD studies

The information relative to the effect of different antimicrobial concentrations on the time-course of microbial killing and suppression of the emergence of resistance can be achieved *in vitro* through static time-kill experiments and dynamic models of infection (Singh & Tam 2011).

Static time-kill experiments investigate the effect on the pathogen exposed to fixed antimicrobial concentrations at differing MICs and enable the preliminary study of the bacterial growth and killing dynamics, the effects on the starting bacterial inoculum and the probability of resistance emergence along drug exposure. However, these studies do not represent the clinical scenarios (Bergen et al. 2011).

On the other hand, dynamic models, address this main limitation of static studies while exposing the bacteria to drug changing concentrations along the course of therapy to better mimic for the *in vivo* PK/PD profile. The microorganisms are exposed to antimicrobials in reaction vessels and perfused continuously with fresh media to promote growth and remove metabolic waste. The vessels can be repeatedly sampled to monitor growth-kill kinetics and drug resistance in relation to the drug exposure over time. Dynamic models can be tailored to investigate specific clinical scenarios, such as medical-device associated infections, biofilms, co-culture with human epithelial cells, the effects of human plasma, etc (Gloede et al. 2010). Hollow fibre systems are one of the most widely employed techniques, whereby microorganisms are contained in a cartridge of capillaries over which drugs, nutrients and waste products can freely diffuse. They also allow to mimicking specific population PK profiles with differing organ function and maturation (e.g renal function) and its linked PD effects (Bergen et al. 2016).

In vivo PK/PD studies

Animal infection models have been used in the therapeutic evaluation of antimicrobials for decades. In contrast to *in vitro* studies, they provide the opportunity to study drug efficacy while accounting for the host immune response and the pathogen virulence *in vivo* (D. Andes 2002). They also enable the investigation of the PD effects at the site of infection (e.g the lung). Most of the *in vivo* PK/PD studies have been performed in small rodents (mainly mice) and rabbits. Studies can also be designed to mimic a specific infection syndrome, such as pneumonia, a BSI or meningitis (Docobo-Pérez et al. 2012). Furthermore, multiple endpoints can be used in assessing the PD: survival/mortality, bacterial counts, biomarkers, pro-inflammatory cytokines, etc. Nonetheless, the establishment of an

infection may require the animals to be rendered neutropenic by prior administration of an immunosuppressant (e.g cyclophosphamide)(Zuluaga, Salazar, C. a Rodriguez, et al. 2006).

The main challenge or limitation of small animal models is that the PK profile can be very different to that observed in humans due to much faster drug clearances. Therefore, careful experimental design with respect to doses, frequency and method of administration is required (D. Andes 2002). Many logistical and ethical considerations are additional requirements and the principles of the "three Rs" (replace, reduce and refine) need to be implemented to ensure good science and the support of the public for the use of animals in research (Kilkenny et al. 2010).

PK/PD population mathematical modelling

The use of PK/PD mathematical modelling has allowed for the accurate and robust analysis of the PK/PD relationships, together with the maximisation of clinical data. It is carried out to identify and quantify PK/PD variability in the population (e.g. *in vitro* experimental hollow-fibre cartridges, animal participants and/or patients) and to record the experience from that collection of patients/subjects in the form of a population model that can be subsequently used as prior information (Bayesian prior) to predict the drug´s behaviour or the PK/PD relationships in a similar and larger population of patients/subjects, as well as in the individuals receiving the same drug (Jelliffe et al. 2000).

Population analysis allows for sparse sampling from each individual because information is shared within the population. In a PK model the simplest structural model includes one compartment and two PK parameters, typically the volume of distribution and clearance. However, more complex structural models, e.g. including two compartments and four PK parameters plus additional PD parameters to describe

the time course of the microorganism growth-kill dynamics, are often required to accurately describe changes in PK/PD relationships observed over time in subjects.

There are parametric and non-parametric methods for mathematical population modelling (Bustad et al. 2006). The parametric methods allow separating inter-individual variability in the population from the intra-individual variability in the individual subjects, and from the variability caused by the assay error. Examples of parametric methods are: the standard two-stage approach, the iterative two-stage Bayesian method and the non-linear mixed effects modelling method (NONMEM) developed by Sheiner and Beal (Boeckmann et al. 2011). The non-parametric modelling methods do not make assumptions about the shape of the parameter distributions (e.g Gaussian, multi-modal). They can detect unsuspected subpopulations of subjects and obtain multiple parameter estimates, basically one for each subject (Jelliffe et al. 2000). The main weakness is that they cannot separate the various sources of variability into their respective components. Examples of non-parametric methods are: non-parametric expectation-maximisation (NPEM) and non-parametric adaptive grid (NPAG), both of which use exact computation of the likelihood (Bustad et al. 2006).

Once a population PK and/or PK/PD model has been developed, Monte-Carlo simulations are the most widely used statistical strategy to compute concentration-time profiles for a larger population to explore further the PK/PD variability and/or the proportion of patients/subjects reaching a certain PD target (Tam et al. 2006). However, the use of a fixed target has limitations since the whole distribution of the time-course of bacterial killing and resistance emergence is not considered.

In conclusion, PK/PD mathematical models can combine available knowledge of the exposure-effect relationships of antimicrobials, which may be

based on *in-vitro*, animal or clinical data, with clinical population specific PK data. This enables, for instance, bridging the information from the bench to the bedside, by allowing the study of more safe and effective candidate dosing regimens in the most targeted clinical setting (e.g preterm neonates with haematogenous *Candida* meningo-encephalitis) (Hope & Drusano 2009).

1.4.3-Clinical PK studies in children: features and regulations

Children present unique challenges and demands for the conduct of high quality clinical trials. Rich PK sampling in critically ill and/or low birth weight infants may be unfeasible and difficult to justify. Rapid changing physiological, pathological and developmental variables may also be captured through repeated sampling, the use of well-validated biomarkers and target specific studies. Statistical methods such as D-optimal design theory can be used to define the most informative sampling times throughout the dosing interval while minimising the number of samples needed (Tam et al. 2003). The use of dried blood spots and ultra-low sample volumes can also minimise this issue. In addition, PK/PD analyses and bridging the experimental findings to humans, both adults and children, allow to study the right dose at the first time in clinical studies, which also facilitates and decreases the number of patients recruited to trials (Hope & Drusano 2009).

In the last decade, both the FDA and the EMA have developed strategies to facilitate the prompt and safe investigation of medicinal compounds in children and neonates. For instance, they both support the extrapolation of information from adults to children provided there are adequate safety data in the latter and the pharmacodynamics can be assumed to be the same in both populations (Steinbrook 2002; European Medicines Agency 2011). This approach requires the development

of robust population PK models in both adults and children, which enables the design of regimens, that allows drug exposures in both populations to be matched. However, the safety of a drug cannot often be extrapolated from adult data, and the course of the disease and the PD are not always the same between adults and children, not even between neonates and young infants and older children and adolescents. A paediatric investigational plan (PIP) is now a pre-requisite by the EMA at the end of Phase I studies of new compounds for the approval of new agents, thus the careful design of paediatric PK/PD studies that incorporate risk proportionate approaches have become mandatory in the development of new antimicrobial compounds (European Medicines Agency 2011).

1.5- PHARMACOKINETICS-PHARMACODYNAMICS (PK/PD) OF STAPHYLOCOCCAL BLOODSTREAM INFECTION MAINSTAY OF THERAPY AGENTS: VANCOMYCIN AND TEICOPLANIN CURRENT PAEDIATRIC DOSING RATIONALE

1.5.1- PK/PD of Vancomycin

Introduction: indications and use

Vancomycin is a glycopeptide agent, introduced into clinical medicine in 1956 for the management of severe Gram-positive bacterial infections, particularly the advent of penicillin-resistant and later on, methicillin-resistant staphylococcal infections (Levine 2006). It is a high molecular weight molecule that inhibits the cell wall synthesis of Gram-positive bacteria by the formation of stable complex murein pentapeptides, thus causing inhibition of further peptidoglycan formation.

Vancomycin came into disuse in the 1960s because of adverse effects, such as, generalised skin eruptions, phlebitis and more importantly, renal failure and deafness. After the purification of the compound and thus, the improvement on its safety profile, it became again increasingly used in the 1980s over the next three decades due to the widespread appearance of resistant pathogens, namely MRSA, methicillin-resistant CoNS and penicillin-resistant *Streptococcus pneumoniae*. It also became the drug of choice, as an oral agent, for pseudomembranous enterocolitis (Fekety & Shah 1993; Levine 2006).

The practice of vancomycin routine TDM has been a matter of intense debate for many years due to conflicting evidence in relation to the current use of serum drug concentrations to predict and prevent drug-induced toxicity, and as a proxy measure of efficacy in treating infections. Currently, the IDSA recommends to monitoring only troughs, and not peaks, just before the fourth dose in patients with

normal renal function. Concentrations are recommended to be maintained > 10 mg/L to avoid development of SA resistance and to achieve a 15-20 mg/L range for effectiveness, as such range would achieve an AUC/MIC ratio ≥ 400 in most patients if the microorganism MIC is ≤ 1 mg/L (Rybak et al. 2009). However, this recommendation is based on limited clinical evidence data from adult patients with invasive MRSA infection (Moise-Broder et al. 2004).

The concept of introducing PK/PD knowledge into clinical practice marked an important milestone into vancomycin therapy. However, there are remaining questions around the efficacy and safety of this approach, the optimal dosing and the monitoring of vancomycin in special populations-including children and neonates- and also, against a variety of Gram-positive microorganisms with differing susceptibility patterns.

Pharmacokinetics

The PK profile of vancomycin has been characterized by either one, two or three- compartment PK structural models. It is administered intravenously for systemic therapy with a standard infusion time of at least 1 hour to reduce adverse events (Rybak 2006). Its pharmacokinetic profile is summarized as follows:

In patients with normal renal function, it has a α-distribution phase of approximately 30 minutes to 1 hour and a β-elimination half-life of 6 to 12 hours. The volume of distribution is 0.4-1 L/kg (Rybak 2006). Its protein binding has been assumed and predicted to be approximately 50% and 41.5%, respectively, but considerable variability has been reported (Butterfield et al. 2011).

It is eliminated primarily unchanged by glomerular filtration (80-90%) and the remaining amount by non-renal mechanisms that remain unknown. Mean total body clearance is around ranges of 0.71-1.31 ml/min/kg (Matzke et al. 1986).

In neonates, due to the developmental characteristics, a larger volume of distribution and decreased total clearance has been described, compared to older children and adults. Average volume of distribution at steady state in term neonates ranges from 0.57 to 0.69 L/kg, and 0.38-0.97 L/kg in pre-term infants, although very few studies are available. Mean elimination half-life times in neonates of varying gestational and post-natal ages are around 3.5-10 hours, reflecting lower values for clearance. Clearance values vary between 0.63 ml/kg/min (0.038 L/kg/h) and 1.4 ml/kg/min (0.084 L/kg/h)(De Hoog et al. 2004).

Vancomycin penetrates into the majority of body spaces, although the concentrations achieved are variable and dependent, to some extent, to the degree of tissue inflammation. For instance, vancomycin CSF penetration was significantly higher in the meningitis group (serum to CSF ratio=48%) than in the group without meningitis (serum to CSF ratio=18%)(Albanèse et al. 2000). Similarly, penetration into the lung is highly variable and reports differ between patients without inflammation (14%) and critically ill patients with ventilator-associated pneumonia (24%) (Lamer et al. 1993).

Pharmacodynamics

A number of *in vitro* and animal studies have been performed to determine the relationship between vancomycin concentration and killing activity. Initially, most *in vitro* killing curve experiments evaluating fixed vancomycin exposure concentrations as small increments of the MIC against *S. aureus* and *S. epidermidis* demonstrated that killing activity does not change as a function of increasing concentration (2 to 64 x MIC) and that the best predictor of vancomycin efficacy was the fraction of time that the concentrations are above the MIC (%T/MIC)(Löwdin et al. 1998). In a dynamic *in vitro* model mimicking the adult elimination half-life of

vancomycin (6 hours), the stepwise increasing concentrations of vancomycin (5-40 mg/L) did not affect the rate or extent of bacterial killing against SA (Larsson et al. 1996). *In vitro* time-kill curves using concentrations between 2-50 mg/L in the logarithmic phase of growth of 12 isolates of CoNS, did not show either any correlation between killing rates and vancomycin concentration (Ackerman et al. 1992).

On the contrary*, in vitro* and neutropenic mouse thigh infection models have determined that the area under the concentration curve divided by the MIC (AUC/MIC) is the best predictor of the activity of vancomycin against methicillin-susceptible S. aureus (**figure 1.3**). Furthermore, in a *Streptococcus pneumoniae* and *SA* non-neutropenic mouse peritonitis model, it was demonstrated that the peak serum concentration divided by the MIC (peak/MIC) and the AUC/MIC were the pharmacodynamic indexes with the most predictive value (Jenny Dahl Knudsen et al. 2000). More recently, the concentration and time-dependent killing of vancomycin (AUC/MIC driven activity) was confirmed against MRSA infection in an *in vitro* dynamic HFIM with once daily dosing of vancomycin (Nicasio et al. 2012).

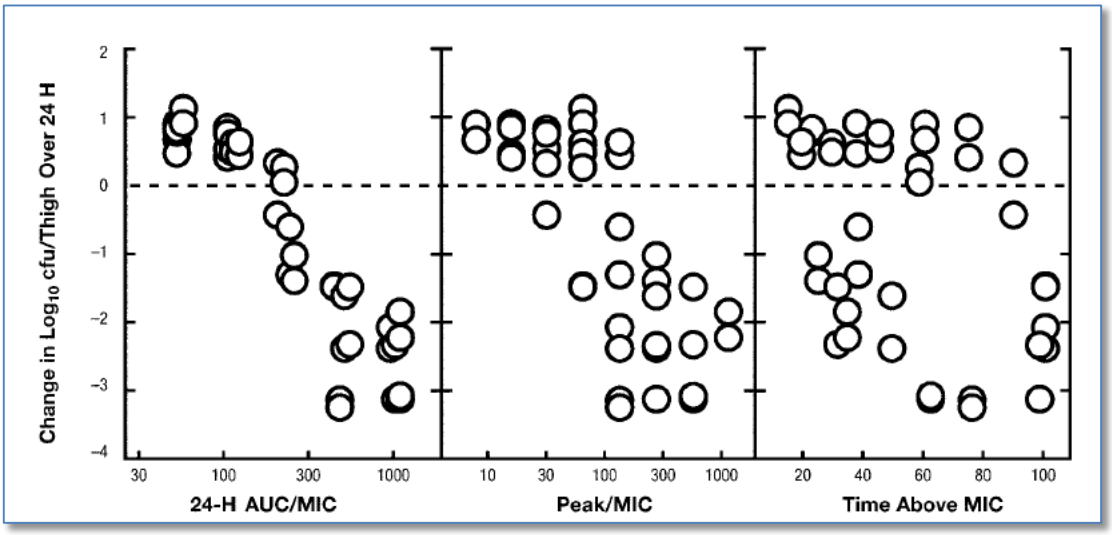

Figure 1.3- Relationship between PK/PD indices for vancomycin and bacteriological efficacy against MS-SA.

The plot illustrates the change in colony-forming units (CFU) in an experimental mouse thigh infection model along the drug exposure in three different ways. It shows that AUC/MIC is the most relevant PK-PD index for predicting efficacy against MS-SA. Data are taken from (Rybak 2006).[Original unpublished work from Ebert S et al. Abstract-439. Program and abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy (New York). Washington, DC: American Society for Microbiology, 1987:173. 24].

Paediatric Clinical Vancomycin PK studies and rationale for current dosing regimens

The current European Society for Paediatric Infectious Diseases (ESPID) Manual of Childhood Infections-"The Blue Book"- recommends the following dosage regimens in neonates and children: 1) neonates < 29 weeks postmenstrual age (PMA): 15 mg/kg every 24 hours; 2) neonates 29-35 weeks PMA: 15 mg/kg every 12 hours and 3) neonates > 35 weeks PMA and older children: 15 mg/kg every 8 hours, all doses adjusted according to the recommended trough concentrations (Sharland et al. 2016). A dose of 15 mg/kg every 6 hours (60 mg/kg/day) is

recommended by the IDSA in children (Liu, Bayer, Cosgrove, Daum, Fridkin, Gorwitz, Kaplan, Karchmer, Levine, Murray, Rybak, D. a. Talan, et al. 2011). In addition, the BNFC recommends trough concentrations of 10-15 mg/L for most indications and 15-20 mg/L for less sensitive strains of MRSA (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015b). This vancomycin dosing for neonates, however, is based on expert opinion and not on clinical trial data. For instance, de Hoog M et al. reported 17 different dosing regimens being used in pre-term neonates (De Hoog et al. 2004).

Vancomycin administered as an intermittent infusion is one of the most studied antibiotics via population PK modelling. Many studies have reported vancomycin PK parameters in children and neonates, using concentrations that have been mainly obtained from routine therapeutic drug monitoring. They have also investigated factors influencing inter-individual variability (e.g body-weight/size, age and renal function) and have proposed optimized dosing regimens that have not been investigated prospectively and uniquely internally validated (Seay et al. 1994; Grimsley & Thomson 1999; De Hoog et al. 2000; Lamarre et al. 2000; Capparelli et al. 2001; Kimura et al. 2004; Mulla & Pooboni 2005; Anderson et al. 2007; Allegaert & Anker 2007; Lo et al. 2010; Marqués-Miñana et al. 2010). Nonetheless, results of these studies have not been compared in a randomized clinical trial for effectiveness and consensus on optimal dosing has not been reached in clinical practice.

Similarly to teicoplanin, in neonates and children, there is no evidence data relating serum vancomycin concentrations to effect. Few studies have evaluated whether current dosing can actually achieve the recommended IDSA vancomycin PK/PD optimal target AUC/MIC > 400. For instance, using Monte Carlo Simulation with clinical PK published data from children, AUC/MIC predictions were

calculated across a range of doses (40-60 mg/kg/day), suggesting that, even when the microorganism MIC is 1 mg/L, a dose of 40 mg/kg/day did not achieve the target. Even at the highest recommended dose of 60 mg/kg/day, the PK/PD target was neither achieved when the microorganism MIC was 2 mg/L (Frymoyer et al. 2013). On the other hand, a retrospective study examining neonatal vancomycin PK data, showed that for the majority of patients studied, a trough > 10 mg/L was adequate to achieve the PK/PD target AUC/MIC > 400. However, the authors highlight the difficulty of achieving consistently target exposures in this population age-group given the high clinical and PK variability (Frymoyer et al. 2014).

In summary, the definition of optimal vancomycin PK/PD targets against staphylococcal infections and the optimal dosing to achieve those remains to be elucidated from pre-clinical and clinical studies in the paediatric population. In addition, uncertainty about vancomycin-induced nephrotoxicity and otoxicity still remains and although rare, a clear relation between serum concentrations and toxicity has not been demonstrated (De Hoog et al. 2004). Furthermore, given the high population heterogeneity, in order to make a significant clinical and microbiological outcome impact, a combination of strong PK/PD evidence together with robust dosing support tools to help clinicians with dose and monitoring individualization, will be likely needed.

1.5.2- PK/PD of Teicoplanin

Introduction: Indications and use

Teicoplanin is a lipoglycopeptide agent that has been marketed in Europe by Sanofi-Aventis Ltd. under the trade name of Targocid® since 1989 (The Electronic Medicines Compendium 2014). It acts by binding to the outer layers of bacterial peptidoglycan before binding to the terminal amino acyl-D-alanyl-D-alanine precursor preventing peptidoglycan elongation, therefore, disrupting the bacterial cell wall synthesis. Teicoplanin is bactericidal against the majority of aerobic and anaerobic Gram-positive bacteria (A. P. R. Wilson 2000).

Its use, together with vancomycin, has increased rapidly in the last decade due to the rise of methicillin resistant staphylococcal infections. With a similar mode of action to that of vancomycin, teicoplanin displays a better safety profile. It has been shown in two extensive systematic reviews and meta-analysis that teicoplanin is less nephrotoxic than vancomycin (Svetitsky et al. 2009; Cavalcanti et al. 2010). This favourable safety profile together with a distinct pharmacokinetic profile, with a longer half-life that allows once daily dosing, makes it very suitable for the treatment of serious Gram-positive bacterial infections in neonates and children (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015a).

Nonetheless, a number of issues have prevented a more extensive use of teicoplanin, in comparison to vancomycin, worldwide. The first one relates to the composition and synthesis of the compound. Teicoplanin is derived from the actinomycete *Actinoplanes teichomyceticus* and it is a mixture of 5 major components (A2-1, A2-2, A2-3, A2-4, A2-5), a hydrolysis component (A3-1) and 4 minor components (RS-1 to RS-4). The A3-1 component is the core glycopeptide that is common to all teicoplanin-like compounds (Bernareggi et al. 1992). This

complexity on its structure reflects the main concerns with respect to the composition and disapproval by regulatory authorities of teicoplanin generic products, as well as their potential impact on the pharmacodynamics (The European Medicines Agency 2010). Another issue relates to measuring teicoplanin concentrations in clinical samples. A number of analytical methods have been used (e.g. Bacillus subtilis bioassay, solid-phase enzymatic receptor assay [SPERA], fluorescence polarisation immunoassay [FPIA] and high-performance liquid chromatography [HPLC]. FPIA has the highest specificity and reliability and is the method of reference (McMullin et al. 1994). The readouts from these different assays differ, thus the conclusions form PK/PD studies are dependent on the analytical method that was used. Finally, there is an important lack of knowledge of the pharmacodynamics of teicoplanin that is clearly represented in the following table summarizing the EUCAST rationale for the selection of teicoplanin clinical breakpoints and its evidence relative to the pharmacodynamics, which is blank. Briefly, until recently, a C_{min} of >10 mg/L (15-20 mg/L for deep-seated infections) has been associated with higher clinical cure rates for most indications than levels of 5 mg/L (Harding et al. 2000). The state of the art with respect to teicoplanin pharmacodynamics will be described in this section.

5. Pharmacodynamics				
AUC/MIC for bacteriostasis				
AUC/MIC for 2 log reduction				
fAUC/MIC from clinical data				
Comments	• There are limited clinical data relating pre-dose (trough) teicoplanin concentrations to clinical outcome. • Two values are given where references differ. Cells are left empty when data are not readily available.			
References	• Harding et al. J Antimicrob Chemother 2000; 45: 835.			

Table 1.2- Rationale for the teicoplanin EUCAST clinical breakpoints, version 2.1 (2010).

http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Rationale_documents/Teicoplanin_rationale_2.1.pdf

The uncertainties concerning the optimal dosage of teicoplanin have discouraged its use in some centres over vancomycin, as well as its license in countries such as the US. This is due to the poor results of early comparative clinical trials and different methodological approaches to those followed in Europe. Initially, low doses of teicoplanin (3mg/kg) in monotherapy for the treatment of staphylococcal sepsis were used with little success (Calain & Waldvogel 1990). Later on, when even higher doses (6mg/kg daily) were used in monotherapy, resulted in inferiority when compared to vancomycin (15mg/kg q12 h) for the treatment of SA endocarditis. The trial was stopped early before significance was reached (Gilbert et al. 1991). Higher dosages of teicoplanin were used in other US small trials (10-30 mg/kg/daily) and resulted in similar efficacy than vancomycin (trough levels > 20 mg/L), but still were considered to be of lower cost-effectiveness and safety to that of vancomycin [unpublished] (Wilson et al. 1994). In European trials, on the other hand, cure rates at 6 mg/kg/day were satisfactory in monotherapy, and were allowed to be given with concomitant agents (amynoglycosides) in more difficult to treat patients (Lewis et al. 1988).

Local use and practice of teicoplanin in Alder Hey Children's NHS Foundation Trust Hospital (AHFT) and The Liverpool Women's NHS Foundation Trust Hospital (LWH) (neonatal unit)

Traditionally, vancomycin was the drug of choice for the treatment of CoNS infections at the Neonatal Intensive Care Unit (NICU) at the Liverpool Women´s Hospital (LWH) until 2003. Since then, teicoplanin has been used as the first line treatment agent for CoNS infections and as second line treatment for empirical late – onset infections due to its safety and ease of administration properties. Regarding the treatment of serious Gram-positive bacterial infections in older children, clinicians at AHFT tend to use more frequently vancomycin over teicoplanin, particularly due to the uncertainty about the evidence behind teicoplanin dosage regimens. However, teicoplanin is used as first line empirical agent in the treatment of central line-associated infections and other potential hospital acquired infections in the pediatric intensive care unit (PICU).

Prescription rates are considerable between both hospitals. In the last 9 years in the Neonatal Unit at The Liverpool Women's Hospital, pre-term and term neonates have been prescribed teicoplanin as second line anti-Gram positive microbial for late onset bacterial infection with percentages over total year admissions ranging from 3.8 (1302 admissions) to 10.1% (1217 admissions) in 2009 and 2007, respectively. That is about 50 to 123 neonates on teicoplanin each year, as compared with 20.7 and 49.5% of patients that received benzylpenicillin (first line therapy) the same years in the same unit (269 to 603 babies per year). In Alder Hey Children's Hospital, teicoplanin is a stock item; assumptions around vial usage per month indicate that a maximum of 150 and 200 doses of 400 mg and 200 mg respectively,

are used per month- assumption made 1 vial equals 1 paediatric dose- (D. Sharpe, personal communication).

Pharmacokinetics

The pharmacokinetic profile of teicoplanin is summarized as follows:

It is not absorbed orally and is not substantially metabolized (only a 2-3% of the drug is metabolized). Intramuscular administration is well tolerated and its absorption is rapid. Systemic availability is nearly 100%. A 6 mg/ kg dose intramuscularly is associated with a peak serum concentration of 12 mg/L after 4 hours compared with 43 mg/L at 30 minutes of intravenous administration in adults (Antony et al. 1991).

The pharmacokinetics of teicoplanin follow a tri-exponential decay, being the α, β and Υ half-lives, 0.4 to 1 hour, 4.7 to 15.4 hours and 83 to 168 hours, respectively. The volume of distribution (V_d) is 0.9 to 1.6 L/kg at steady state. Notably, longer sampling periods (up to three weeks) provide the greater values in the terminal elimination phase (A. P. Wilson 2000).

Teicoplanin is predominantly bound to plasma proteins (around a 90%) and binding appears to be linear with rising concentrations to 300 mg/L (Bernareggi et al. 1991). The protein-binding ability to neonatal serum has also been investigated. At concentrations ranging from 10 to 100 mg/L, the rate of binding was 71.9-80.5%, considerably lower than in adults (Sando et al. 2004).

The kinetics of distribution is not dependent on dose. Being highly bound to serum proteins (albumin), the free fraction is not related to overall serum concentrations. In a study by Del Favero and colleagues in 5 adult volunteers, the values of half-life, volume of distribution at steady state and initial volume of distribution, did not differ significantly after individual doses of 15, 20 and 25

mg/kg, and the AUC (area under the concentration-time curve) increased linearly (Del Favero et al. 1991).

On the other hand, tissue distributions have been shown to be very variable and standard teicoplanin dosages may not achieve desirable concentrations for the MIC of the targeted microorganisms. Teicoplanin concentration in bone diminishes importantly after 48 hours following a single dose, being even less satisfactory in cartilage. It does not reach the cerebrospinal fluid well but intraventricular administration can be used. Similarly, it has a poor intraocular and fat penetration. However, therapeutic concentrations have been found in wound exudates after intravenous administration (A. P. R. Wilson 2000).

In adult patients with ventilator-associated pneumonia (VAP), a high dose regimen of 12 mg/kg of teicoplanin, proved to reach target unbound trough serum concentrations above 2 mg/L (corresponding to a target trough total serum concentration of approximately 20 mg/L), which were similar to lung epithelial lining fluid concentrations (ELF). Therefore, the authors showed an adequate lung penetration of teicoplanin at high doses, in a small sample size of 13 critically ill participants (Mimoz et al. 2006).

Teicoplanin is eliminated via the kidneys, being most of the drug eliminated in the terminal phase. Clearance of the unbound drug is by glomerular filtration and both tubular reabsorption and renal secretion are minimal. Renal clearance has been between 8.1 and 10 ml/h/kg in various studies and total body clearance has been reported to be 11 ml/h/kg. The terminal phase half-life has been reported to be around 168 hours with conventional doses in healthy volunteers (Antony et al. 1991). However, at high multiple doses (30 mg/kg), the volume of distribution has been noted to decrease significantly, with no change in clearance and therefore, a decrease

in the terminal half-life around 96 hours, probably due to a saturated process of tissue protein binding (Smithers et al. 1992).

In children, teicoplanin PK have been characterized following a tri-exponential model with a distribution (α) and two elimination phases (β and γ half-lives (Terragna et al. 1988; Reed et al. 1997), but also have been described following the kinetics of a two compartment model (Tarral et al. 1988; Dufort et al. 1996; Sánchez et al. 1999). For more detailed information, please see summary **tables 1.3** and **1.4.**

Pharmacodynamics

Despite its extensive use, there is relatively very few PK/PD data for teicoplanin, compared to vancomycin. Classically, dosage regimens have been determined by the need to achieve concentrations in serum and targeted tissue over the MIC of the majority of likely microorganisms throughout the dosing interval. Nonetheless, clinical trials have demonstrated that higher doses may be required in some deep-seated infections. Currently, a trough (pre-dose concentration) of 15 mg/L (measured by FPIA) is proposed in the summary of product characteristics (SPC) by days 3-5 of therapy for most indications in both adults and children. This has been recently increased to 20 mg/L and 30-40 mg/L for the treatment of deep-seated infections and infective endocarditis, respectively, without changing the recommended dosage regimens (The Electronic Medicines Compendium 2014). These recommendations are based on scant clinical evidence in adult patients and with a poor understanding of the pharmacodynamics of teicoplanin (Matthews et al. 2007; Sato et al. 2006; Ueda et al. 2012).

Pre-clinical teicoplanin PK/PD studies are scarce. A pre-clinical PD study carried out in a mouse peritonitis model caused by *Streptococcus pneumoniae* or SA

suggested, a decade ago, that both fC_{max}/MIC and *%f* T/MIC were the relevant PK-PD indexes describing teicoplanin activity in this model. A maximum peak free drug concentration (fC_{max}) over MIC ratio of at least 2-3 was required for efficacy (J D Knudsen et al. 2000).

Small clinical trials in adults have acknowledged the importance of considering teicoplanin exposure in terms of area under the concentration-time curve (AUC_{0-24}), combined with the patients characteristics (e.g clearance, body weight) to achieve an appropriate microbiological response (Kanazawa et al. 2011). For instance, an AUC_{0-24} of at least 800 mg*h/L was necessary to eradicate or cure MRSA infections (MIC of 1 mg/L) in ICU patients (Hagihara et al. 2012). More recently, an AUC/MIC ratio of ≥ 900 mg/h/L has been suggested for bacteriological response in a study with 46 patients with MRSA infection (Takeda et al. 2016a).

Paediatric Clinical PK studies and rationale for current regimens

In 1993, the UK Association of the British Pharmaceutical Industry (ABPI) data sheet recommended, for children, 3 loading doses of 10 mg/Kg at 12 hourly intervals followed by 6 mg/kg daily with increased maintenance dose to 10 mg/kg in severe infections (based on serum pre-dose concentrations that should not fall below 10 mg/L). For neonates, a loading dose of 16 mg/kg once followed by 8 mg/kg thereafter every 24 hours is recommended. Current British National Formulary for Children (BNFC) (2015) dosage recommendations remain the same after twenty years, and they are based on scarce evidence data (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015b). In addition, due to this lack of safety and efficacy data, teicoplanin is not licensed in the EU for the use in neonates and infants < 2 months of life.

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Table 1.3- Classical Teicoplanin Pharmacokinetic Studies

Reference	Population	Sample size Dosage regimen	Methodology	Results
(Lemerle et al. 1988) "Teicoplanin in combination therapy for febrile episodes in neutropenic and non-neutropenic paediatric patients"	Children with neutropaenia with severe suspected or proven Gram-positive infection in combination with other agents for Gram-negative bacteria (mean age 7 yo 2 m)	20 The first 7 patients: 6mg/Kg per infusion (twice the first day, then daily); the 8th patient had twice 6 mg/Kg the 1st day, then 8mg/kg daily; two patients had 8 mg/kg/12h the 1st day, then 10mg/kg daily. The last 13 patients had 10 mg/kg each infusion.	-Serum concentrations were measured on days 1,2,5,9 and 14 and then once a week with a trough assay, and a peak assay one hour after the end of the half-hour infusion. -Each isolate was investigated for sensitivity to antibiotics and MIC of teicoplanin -Serum bacteriostatic and bactericidal activity was measured on day 5 before and 1 hour after the end of infusion. -SPERA assay	-Mean of 7 samples per patient -Each patient had at least two samples taken at expected minimum level and two at expected peak level. -The low dose group (6 mg/Kg) showed a mean trough level of 4.1 mg/L and a mean peak level of 20.5 mg/L. -The high dose group (10 mg/Kg) showed a mean trough level of 11.2 mg/L and a mean peak level of 36.3 mg/L. -Assessment of clinical efficacy was done in 7 patients: 5 cures and 2 failures, being the failures in the low dose group. -All failures are not explained by PKs as in all cases serum concentrations were above the MIC. -The authors recommend the dose of 10 mg/Kg daily with a second loading dose the first day for children with neutropaenic fever.
(Terragna et al. 1988) "Pharmacokinetics of teicoplanin in paediatric patients".	Children (2-12 yo), male, requiring prophylaxis against Gram-positive bacteria before minor surgery or for medical indications. (weight: 13-32 kg)	13 Single dose of 3mg/kg iv (3-5 min infusion) for prophylaxis and 6mg/kg every 12h first day and then, daily for 4 patients for treatment.	- Blood samples (3ml) taken just before dosing and at 1,3,6,12,24,48,72,96,120,144,168 and 192h after dosing in the prophylaxis group. -Blood samples taken at 5min, 1,3,6 and 12 h after first administration and just before each subsequent dose up to 15 days in the treatment group. -Sampling time 13 days in the prophylaxis group. -Urine was collected for 8 days post-administration. -Open Tri-compartment model to the individual data and a non-compartmental analysis. -Microbiological assay for plasma and urine.	-No adverse events were reported. Local and systemic tolerability were good. -Cmax (1h) (non-compartmental analysis): 14.79±0.82 mg/L -AUC (mean): 207.98 mg.h/L -CL=0.0148 L/h/kg; -V(terminal)1.25L/kg; Vss: 0.80 L/Kg ; terminal half-life 58h -These main PK parameters are similar to those obtained from adult volunteers with same dose and assay methodology except for C and volume of the central compartment. Authors relate these

			-Age and weight were analysed as covariates by linear regression.	differences to fewer experimental points in first part of the study. -No indication of linear correlation between terminal half- life and age or weight was found.
(Tarral et al. 1988) "Pharmacokinetics of teicoplanin in children"	6 children (4-12yo) hospitalised for severe urinary infection and treated with cefotaxime iv and 4 neonates (3d-25d) in intensive care and treatment with mezlocillin and netilmicin.	10 (6 children and 4 neonates) Single dose of 6mg/kg iv over a 10-min infusion in children and 20 min infusion in neonates.	-Blood samples taken at 10 min and 1,4,12 and 24h after the end of infusion, up to ten days. -Total 24h collection in children. -Serum and urine teicoplanin concentrations determined by HPLC. -Open two-compartmental model for serum concentrations.	- In children: -AUC: 221.64 μg/h/ml -Vc: 0.1286 L/Kg -Vss: 0.5432 L/kg -CLT: 28.19 ml/h/kg -In neonates: -AUC: 392.93 μg/h/ml -Vc: 0.3073 L/kg -Vss: 0.6139 L/kg -CLT: 15.78 ml/h/kg -In summary: -Trough serum concentrations at 24h were too low for MIC (2.04 mg/L). They recommend 10 mg/kg/day for children and 6mg/kg/day for neonates, who had lower elimination rates and clearance parameters.
Peller et al (1993) "Safety and efficacy of teicoplanin in the treatment of Gram-positive infections in paediatric patients in Germany"	Children hospitalised (2m-15 yo) with suspected or proven Gram positive bacterial infections	12 children got serum concentrations of teicoplanin assayed (out of 51) LD: 10 mg/kg every 12 h x 3; MD: 10 mg/Kg in severe infections/ 6mg/kg in mild-moderate infections every 24h (iv infusion 10-30 min)	-Open multicentre study of efficacy and safety of teicoplanin in hospitalized children. -Microbiological confirmation in 31 patients. -No PK/PD modelling was applied. -Method of teicoplanin assay not reported.	-After the second dose, mean peak levels ranged between 40-70 mg/L and mean trough levels were 10-20 mg/L. -Teicoplanin was considered to be very effective (83% cures and 17% improvements with no failures of therapy) and well-tolerated for the treatment of Gram positive bacterial infections.
(Kacet et al. 1993) "Teicoplanin and amikacin in neonates with Staphylococcal infection"	Neonates with proven staphylococcal infections	52 (mean GA: 30.8 wks) Post-natal age (0-3 months) (mean birth weight 1400g)	-Efficacy study of the combination of teicoplanin+ amikacin in the treatment of severe staphylococcal infections. -Teicoplanin serum levels were measured from samples taken after the first, fifth and last dose	-Peak and trough levels were higher in Group 2. -Trough after the 1st and 5th dose in Group 1 were often low compared with the MICs of the infecting organism,

		Group 1: LD: 10 mg/kgx1, then 6mg/kg daily; Group 2: LD: 15mg/kg x1, then 8 mg/kg daily. Combined with amikacin (30 min infusion)	(at the end of infusion for peak levels and immediately before for trough levels). -Dose was changed into a higher dose in Group 2 after reviewing first 30 patients. -Sampling time (mean): 14 days -No PK/PD modelling was performed.	whereas concentrations in Group 2 were satisfactory. -Tolerance was good in both groups. -Clinical and bacteriological outcomes were similar in both groups.
(Dufort et al. 1996) "Teicoplanin pharmacokinetics in paediatric patients"	Children 3m to 15 yo with febrile neutropaenia that underwent BMT	21 divided into 2 groups A(n=9), B(n=12) A: LD 10 mg/kg x 3 at 12 h interval+ MD 10 mg/kg every 24 h B: same LD+ MD 20 mg/kg every 24 h+ ceftazidime or aztreonam (1h infusion)	-Patients monitored from the 2nd day after start of treatment and periodically thereafter. -Doses were individualized according to plasma levels attained by a bi-compartmental simulation computer program -FPI -Sampling times: peak at 1.5h post-dose, a value at 3h in the beta-phase of elimination and trough 1 h before next dose.	-No significant differences in the PK profiles according to three age groups (<2y;2-12y;>12y) A: trough levels <10 mg/L were not reached in 5 out of 9 patients B: all attained trough levels >10 mg/L -Tolerance was excellent -Mean parameters (SD): -VC: 0.19±0.044 L/kg -CLT: 28.8±9.45 ml/h/kg -AUC: 223±19µg/h/ml -$t_{1/2}$: 21.4±4.9 h
(Reed et al. 1997) "The pharmacokinetics of teicoplanin in infants and children"	Infants and children 2.4-11 yo following and elective surgical procedure	12 6mg/kg iv once daily for 5 consecutive days over 20-30 min infusion	-Blood sampling over 6 days -Multiple timed blood (1.5-2ml) at 20-30 min, at 45 min and at 1,2,6,12 and 24 h after the beginning of first dose; 1,4,8 h after start of second dose; 1,8 and 24 h after start of 3rd dose; 1 and 12h after 4th dose and 0,30,45 min and 1,2,6,12 and 24 h after the start of 5th dose + additional blood samples. -Urine over first 24h was collected. -HPLC and microbiological assay. -Three-compartment PK analysis.	-A trend identified between teicoplanin body CL (decreasing) with increasing age. -No statistically significant associations with age. - Mean parameters: -VdSS: 0.56; $t_{½}$ 16.1; CLt: 36.5. -A dose of 8mg/kg every 12 h should achieve target serum trough concentrations of 11 mg/L in children. Higher doses may be needed for deep-seated infections and/or endocarditis.
(Degraeuwe et al. 1998) "Use of teicoplanin in preterm neonates with Staphylococcal late onset neonatal sepsis"	Preterm neonates 26-32 wks, postnatal age 5-47 days, Birth-weight 570-	23	-Open, non-randomized descriptive study -Individual Pk during multiple-dose iv infusion with peak and trough levels -FPI	-Not PK analysis possible due to inaccurate dosing -32% had trough levels <10 mg/L -90% microbiological and clinical cure

	1740 g	LD 15 mg/Kg x1, followed by MD 8mg/kg every 24 h+ iv gentamicin		in Gram positive septicaemia
(Sánchez et al. 1999) "Teicoplanin pharmacokinetics in critically ill paediatric patients"	Children (7d-12yo) *the two neonates (7 and 10 d old) admitted to PICU with nosocomial infection. Weights: 2.7-40 kg	21 LD 10 mg/kg x3, at 12h interval, followed by MD 10 mg/kg once daily x 7days	-Blood extracted at 30 min, 2.6h and 12 h after first dose and before the next dose after 24h, 48h and 72h and on the 7th day of treatment. -HPLC -Compartmental PK analysis	-No significant differences in the mean serum concentrations and PK parameters between <3m,3-12m and >12m age groups+ from the two neonates. -AUC: 224.5 mg/l/h -11% of trough levels were >10mg/L

AUC: Area under the concentration-time curve; C, CL: Clearance; Cl creat: creatinine clearance; Cmax: Concentration maximum; CLT: Total clearance; FPI: Fluorescence polarization Immunoassay; HPLC: High-performance liquid chromatography; LD: Loading dose; MD: Maintenance dose; MIC: Minimum inhibitory concentration; PICU: Paediatric Intensive Care Unit; SD: Standard deviation; SE: Standard error; SPERA: Solid Phase enzyme receptor assay; t½: half-life time; V: Volume of distribution; Vc: Volume of distribution (central compartment); Vss: Volume of distribution at steady state. QMS: Quantitative microsphere system.

Table 1.3- Classical Teicoplanin Pharmacokinetic Studies.

Table 1.4- Population Teicoplanin Pharmacokinetic Studies

Reference	**Population**	**Sample size** **Dosage regimen**	**Methodology**	**Results**
(Aarons et al. 1998) "Plasma and tonsillar tissue pharmacokinetics following intramuscular administration to children"	Children (3-12 yo) with medical indications for the surgical removal of tonsils. Weights: 12.8-39.8 kg	37 in7 groups 1 y 2: 5mg/kg x1 3: 5mg/kg x2, 12h apart 4 y 5: 5mg/kg x3, 12 h apart 6 y 7:10 mg/kg x1 All doses were provided im and two formulations were used. Drug was used prophylactically.	-Up to 2 blood samples and 1tissue sample were taken after the 1st administration of teicoplanin -Receptor-antibody sandwich assay -A one-compartment open disposition model with first order absorption from the im site used to describe the plasma-concentration-time profile -Population mean parameters and variances were determined by fitting the model to the plasma data by a maximum likelihood method employing a modified EM algorithm. -Model then fitted to tonsillar data by nonlinear least squares.	-Population mean estimates of CL/F and V/F were 0.024/h/kg and 0.61/kg with very little inter-individual variability, 23% for each parameter. -68% of population predictive value for Cmax/dose was 4.6-7.6 mg/L following administration of a 5mg/kg dose to a 20kg child. -Dose, body weight or formulation had no further influence on the PK parameters. -Following 5mg/kg dose to a 20kg child, the Cmax/dose was 3.9 mg/L -Terminal half-life 17.6h -Tonsillar tissue half-life=0.95h
(Lukas et al. 2004) "Pharmacokinetics of teicoplanin in an ICU population of children and infants"	Children (4m-12 yo) admitted to the PICU with developed Gram+ infections Weight:4-28 Kg	20 A: LD 10 mg/kg x3, 12 h apart + 10mg/Kg every 24h B: LD same + 15 mg/Kg every 24h (1 h iv infusion)	**-**Open, randomized and prospective design. -Sampling period: 1h before 1St, 3rd and 5th maintenance dose+ 1 and 3 h after each of those doses. (6 samples per subject)	-Appreciable but non-statistical differences in PK mean and median parameters between the two age groups (<12m and >12m) -Teicoplanin basic bi-compartmental population parameters were (SE): CL: 0.23 l/h (0.12) V1: 3.16 l (0.46); CV% 52% (26%) V2: 4.7 L (0.41); $T_{1/2}$ α, β 2.0 and 79.3 h

				-The frequency of Cmin < MIC for SA was higher in older children -Age dependence in PK covariate analysis.
(Zhao et al. 2015a) "Population pharmacokinetics and dosing optimization of teicoplanin in children with malignant haematological disease"	Children with malignant haematological disease Age (0.5-16.9 yo) Wt: 7.7-90.6 kg	N=85 Received current recommended teicoplanin dosing regimen: 10 mg/kg q12 x3, then 10 mg/kg q24 h	-TDM and opportunistic blood samples (n=143 available for analysis) -QMS (immunoassay) using CDX automate for teicoplanin determination -A 2-compartment PK model with first order elimination was developed with NONMEM	-n=41 children had sub-therapeutic teicoplanin levels (Cmin< 10 mg/L) -CL=0.491 L; Vc=12.9 L -Bodyweight and CLcreat were the significant covariates -For a target AUC of 750 mg·h/L, doses need to be increased to 18 mg/kg in infants, 14 mg/kg in children and 12 mg/kg in adolescents
AUC: Area under the concentration-time curve; C, CL: Clearance; Clcreat: creatinine clearance; Cmax: Concentration maximum; FPI: Fluorescence polarization Immunossay; HPLC: High-performance liquid chromatography; LD: Loading dose; MD: Maintenance dose; MIC: Minimum inhibitory concentration; SD: Standard deviation; SE: Standard error; SPERA: Solid Phase enzyme receptor assay; t½: half-life time; V: Volume of distribution; Vc: Volume of distribution (central compartment); Vss: Volume of distribution at steady state. QMS: Quantitative microsphere system.				

Table 1.4- Population Teicoplanin Pharmacokinetic Studies.

As summarised in the tables above, there is a wide heterogeneity of PK results for children and neonates. Some classical PK studies, have failed to show a correlation between the PK parameters determined and age or weight. Other authors, conversely, have identified some trends of a relationship with age. For instance, Reed and colleagues suggested that drug clearance decreased with increasing age (Reed et al. 1997). However, they all state the need for further studies to better define the influence of patient clinical characteristics in teicoplanin disposition, especially in neonates and infants. In more recent population PK studies, different dosage regimens have been used providing different levels of outcome. For instance, Lukas and colleagues showed that a15 mg/kg regimen rather than the recommended 10 mg/kg dose regimen achieved better target C_{min} in infants and children being nursed in the ICU. Nonetheless, there was a wide disparity in different age-populations groups. This was further supported in the Monte Carlo Simulations, particularly for older children (Lukas et al. 2004).

On the whole, population teicoplanin PK studies in children and neonates are very scarce yet and heterogeneity among published reports does not allow drawing definitive conclusions. The variation in the methodology and design (sampling strategy, duration of blood and urine sampling, sample sizes, age-groups, different teicoplanin assays, different PK models) between studies, probably accounts for the differences reported in teicoplanin pharmacokinetic data. However, despite the apparent disposition differences among paediatric studies, in general, they tend to reflect a more rapid clearance and a smaller volume of distribution (except for neonates) than observed in adults. In addition, the selected populations present with intrinsic various physiological alterations that could alter antibiotics disposition. A well-designed PK study in a representative population that account for the extensive

inter-individual variability, with neonates, infants and older children, is still needed in order to develop an evidence-based teicoplanin optimal dosage regimen.

No pharmacodynamic studies of teicoplanin have been conducted in children or neonates.

1.6- PROJECT AIMS

Aim 1: Characterize the preclinical PK/PD of staphylococcal infections (methicillin-resistant CoNS and SA) using current mainstay therapy (teicoplanin and vancomycin) in neonates and children. Define the magnitude of drug exposure targets that are associated with maximum antibacterial effect and suppression of drug resistance.

Aim 2: Characterize the clinical population PK of teicoplanin in neonates, infants and children.

Aim 3: Construct the software (and population PK model) with clinical data for teicoplanin, that can be used as a tool for the individualisation of regimens in neonates and children to achieve optimal drug exposures for desired efficacy and safety.

Chapter 2

General Materials/Patients

and Methods

2.1- ORGANISM: *IN VITRO* IDENTIFICATION AND SUSCEPTIBILITY TESTING

2.1.1-Coagulase-negative Staphylococci

Five clinical strains were recovered from two different neonatal intensive care units (NICUs) in the United Kingdom (Liverpool Women´s Hospital and St. George´s Hospital in London): three strains of *S. epidermidis* (122648, 122761, 121164) and two strains of *S. capitis* (122828, 062012) were used in the hollow fibre infection model and one strain of each species (122648,122828) in the rabbit animal model (courtesy of Dr Timothy Neal-Liverpool Women´s Hospital). These species were chosen on the basis of its prevalence and clinical significance in neonatal infections as well as on the basis of recent outbreaks of *S. capitis* in European NICUs (Becker et al. 2014; Klingenberg et al. 2005; Rasigade et al. 2012). The bacteria were stored at -80º C in Microbank ^{TM} vials (Pro-lab diagnostics, South Wirral, UK).

The isolates were identified using Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) by Dr Neal. The minimum inhibitory concentration (MIC) for the 5 strains was conducted on 10 occasions, in Müller-Hinton (MH) broth (Sigma-Aldrich, UK) using broth microdilution methodology as described by the Clinical Laboratory Sciences Institute (CLSI) and the European Committee of Antimicrobial Susceptibility Testing (EUCAST)(Clinical and Laboratory Standards Institute 2015; European Committee on Antimicrobial Susceptibility Testing 2003). All the strains used were oxacillin resistant also by E-test (Oxoid Limited, Hants, UK).

2.1.2- Staphylococcus aureus

Staphylococcus aureus subsp.aureus, MRSA ATCC® 43300™(ATCC®, Middlesex, UK) was used for all *in vitro* and *in vivo* experiments. Isolates were stored at -80º C in Microbank ™ vials (Pro-lab diagnostics, South Wirral, UK).

The MIC for the strain was determined using EUCAST broth microdilution methodologies on 3 separate occasions by EUCAST Reference Laboratory (European Committee on Antimicrobial Susceptibility Testing 2003). The strain used was oxacillin resistant also by E-test (Oxoid Limited, Hants, UK).

2.2- DRUGS

2.2.1- Vancomycin

Vancomycin hydrochloride for intravenous infusion clinical formulation (Vancocin 500 mg, Flynn Pharma Limited, Dublin, Ireland) was used for both the *in vitro* and *in vivo* infection models. For the preparation of drug containing plates, vancomycin hydrochloride powder (Sigma-Aldrich, UK) was used.

Vancomycin concentration determination

Analyses of vancomycin concentrations in rabbit plasma and MH broth were performed on a Ultra High performance Liquid-Chromatography tandem Mass Spectrometry system (UHPLC/MS-MS) consisting of a Agilent 6420 Triple Quad (Agilent Technologies, Berkshire, UK) in electro-spray positive ion mode (ESI), and an Agilent 1290 series (Agilent Technologies, Berkshire, UK) equipped with a quaternary pump, online degasser, column heater, auto sampler and chiller. The method used a zorbax eclipse plus C18 column 2.1 x 50mm 1.8µ (Agilent

Technologies, Berkshire, UK) and a 20µL injection volume. A standard curve encompassing 0.05–50 mg/L for plasma and 0.1-50mg/L for broth were constructed from a stock solution of vancomycin at 1000 mg/L in methanol further diluted in methanol to obtain the desired concentration (Fisher Scientific, Loughborough, UK). The internal standard (IS) was phenacetin (Sigma Aldrich, Dorset, UK) 0.1mg/L in acetonitrile (Fisher Scientific, Loughborough, UK). The mobile phase was 100% 0.1% trifluoroacetic acid (TFA) in water 0% acetonitrile with 0.1% TFA (v/v) with a gradient profile changing to 20% and 80% respectively over 2 minutes with an overall run time of 3 minutes and flow rate of 0.5 mL/min. The mass transitions were monitored on m/z 724.91 to 1305.4 for vancomycin and 180.1 to 110.0 for IS. The data was acquired and processed using the Mass Hunter B.6.0.0 software package. The CV% for vancomycin was ≤12.9% over the concentration range 0.05-50 mg/L. The limit of detection was 0.05 mg/L for plasma and 0.1mg/L for broth, the intra and inter-day variation was ≤8.3%. *This method and analysis was performed by Joanne Goodwin and Sarah Whalley.*

2.2.2- Teicoplanin

Teicoplanin for intravenous infusion (Targocid 400/200 mg, Sanofi Aventis, Surrey, UK) was used for the *in vitro* and *in vivo* pre-clinical studies. This is also the product used as per standard of care in the National Health System (NHS) and therefore, the same formulation was prescribed to patients recruited to the PK clinical trial.

For the preparation of drug containing plates, teicoplanin hydrochloride powder (Sigma-Aldrich, Dorset, UK) was used.

Teicoplanin concentration determination

A fluorescence polarization immunoassay (FPIA; Thermo Fisher Scientific, Germany) was used for the quantification of teicoplanin concentrations in serum. This is a homogeneous particle-enhanced turbidimetric immunoassay that utilizes the Quantitative Microsphere System (QMS) technology and was implemented on an automated analyzer Abbott Architect ci4100. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody biding sites of the teicoplanin antibody reagent. A concentration-dependent agglutination inhibition curve was obtained with minimum and maximum rate of agglutination at the highest and lowest teicoplanin concentrations, respectively. The limit of quantification (LOQ) was < 3.0 mg/L. The dynamic range was 3-100 mg/L and total precision (inter and intra-day) was < 6%. *This method and analysis was performed by Kevin Padmore.*

2.3- *IN VITRO* HOLLOW FIBRE INFECTION MODEL

A hollow fibre infection model (HFIM) was used to simulate a typical neonatal and paediatric pharmacokinetic (PK) profile in order to study the dynamic response of CoNS and SA to different concentrations of vancomycin and teicoplanin, respectively. In both cases, the basic model consisted of a central compartment circuit (containing 300 mL of MH broth) and a hollow fibre (HF) cartridge (Fiber Cell Systems, USA) for each dosage regimen investigated plus a control arm. The drug was injected into the central compartment using a programmable syringe driver (Aladdin pump, World Precision Instruments, UK) together with a continuous flow of fresh MH broth infused through a pump from a reservoir (205U, Watson-Marlow, UK). The pump was run at a rate that represented the simulated drug clearance (in

mL/h). A second peristaltic pump connected the MH broth from the central compartment to the hollow fibre cartridge (C2011, Fiber Cell Systems, USA) with a rate about ten times higher than the previous rate, which then returned to the central compartment. Finally, the circuit was completed by the removal of waste from the central compartment via the second peristaltic pump in order to maintain and iso-volumetric system. A schematic representation of the HFIM is shown in **figure 2.1.**

The bacteria were grown in the HF cartridge and left untreated for 24 hours to allow the biofilm formation. Each microbial strain was studied in a different experimental run to avoid cross-contamination. Each experimental run consisted of simultaneous circuits (different dosage regimens and a drug-free control system). The final inoculum was confirmed by quantitative culture on MH agar. This inoculum was expected to reach a density of approximately 9-10 $\log_{10}$ CFU/mL 24 hours post-inoculation and before treatment in the HF. A bacterial density of > 10^2 CFU/mL from a sonicated catheter by quantitative broth culture reflects catheter colonization in patients. For quantitative blood cultures, a colony count of microorganisms grown from blood obtained through a catheter hub should be at least 3-fold greater than the colony count from blood obtained from a peripheral vein to best define a catheter-related bloodstream infection (CRBSI)(Mermel et al. 2009). Despite using the IDSA guidelines for the diagnosis of CRBSI for the choice of inoculum sizes, to our knowledge, there is no reported mean bacterial density data from blood cultures in neonates/children to be diagnostic of LOS or CLABSI.

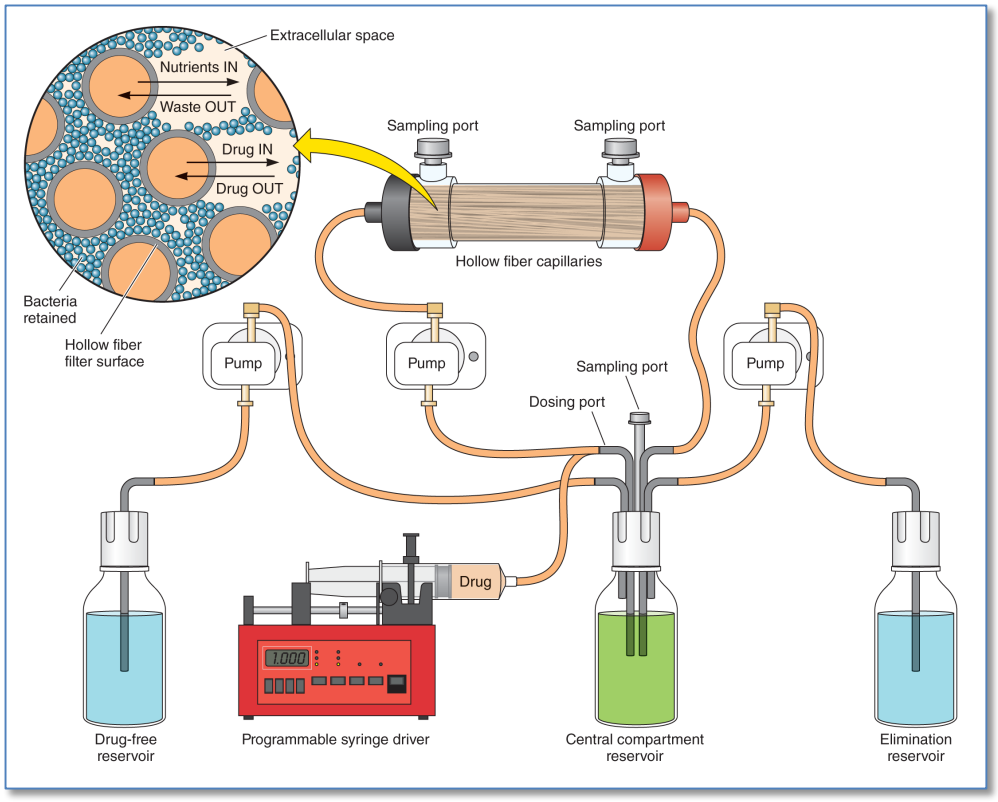


Figure 2.1- Schematic representation of the *in vivo* HFIM.

Illustration made by Patrick Lane (ScEYEnce Studios).

2.4- *IN VIVO* CLABSI RABBIT MODEL

All *in vivo* experiments were conducted under a Home Office project license (40/3630) and approved by the University of Liverpool Animal Welfare Committee. All animals were cared for in line with national guidance in the Biomedical Services Unit (University of Liverpool). A non-neutropenic rabbit model of neonatal CLABSI was developed and used to study the response of CoNS to different regimens of vancomycin (Vancocin 500 mg, Flynn Pharma Limited). Male New Zealand white rabbits (2.68-3.67 kg) were used for all experiments. Two clinical strains of CoNS (*S. epidermidis* 122648 and *S. capitis* 122828) that were obtained from neonates were used for these experiments. Forty-two rabbits were studied (22 rabbits were infected with *S. epidermidis* and 20 rabbits with *S. capitis*). Each experiment

consisted of 6 rabbits (2 controls and 2 different dosage groups of two rabbits each).

A veterinarian established a central venous access with a rabbit jugular vein catheter with Smith´s P.A.S Port® Elite (SAI Infusion Technologies, Illinois, US) under general anesthesia. This enabled the reproduction of a CoNS infection through the central line and subsequent biofilm formation. A schematic representation of the CLABSI model is shown in **figure 2.2.**

A 1 mL volume of 8 $\log_{10}$ CFU/mL was administered via the central catheter of each rabbit and locked with 0.5 mL of lock solution (500 IU/mL of heparin in 10% dextrose). After two hours, up to 0.5 mL of lock solution was removed and the line flushed with 0.5 mL of sterile 0.9% saline. This inoculum was designed to establish a non-lethal model of CLABSI in the rabbits. Each experiment lasted 96 hours. All rabbits were sacrificed 0.5h after the final samples on day 4. At autopsy, the catheters were removed and the tip taken for quantitative culture.

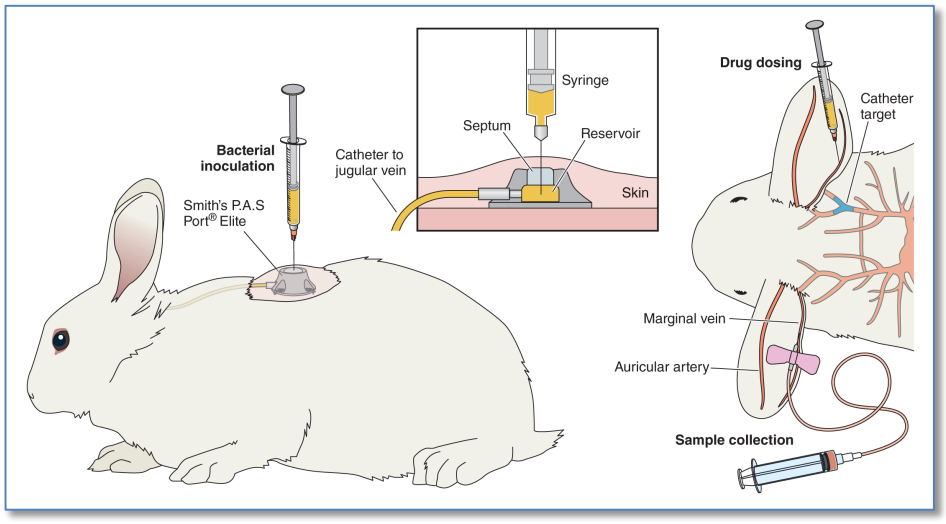


Figure 2.2 - Schematic representation of the in vivo rabbit CLABSI model.

Illustration made by Patrick Lane (ScEYEnce Studios).

2.5- *IN VIVO* MICE THIGH INFECTION MODEL

A neutropenic mice thigh infection model was conducted to evaluate the pharmacodynamic effect of teicoplanin different drug concentrations against an MRSA infection after 26 hours.

Each experiment consisted of 9 male CD1 mice (16-20 grams) that were rendered neutropenic on day -4 and -1 with the intra-peritoneal administration of cyclophosphamide (Baxter, Liverpool, UK) (150 and 100 mg/kg, respectively)(Zuluaga, Salazar, C. A. Rodriguez, et al. 2006). On day 1, mice were inoculated with $2x10^6$ CFU/ml of an MRSA strain (ATCC 43300) in each posterior thigh at a volume of 50 μL. Then mice were evaluated at 2, 12 and 26 post infection, when the appropriate mice were culled and the thighs removed and placed in separate culture tubes with phosphate buffered saline (PBS) for quantitative culture in MH agar.

Teicoplanin therapy was commenced 2 h post-infection and was administered i.v. every 12 hours. Dosages of 2.5, 15 and 100 mg/kg/day were chosen to investigate the total bactericidal effect and the suppression of resistance based on preliminary dose-finding studies. PK sampling was performed at: 0, 0.5, 1, 2, 4 and 12 hours post-dose. Three mice were used per dose-time-point. Samples were immediately spun and plasma stored at -80ºC until analysis.

The health of mice was assessed at hourly intervals and any signs of ill health were recorded. Mice were weighed daily to ensure they did not fall below 20 % of their original weight. In this case, mice were culled.

2.6- C-REACTIVE PROTEIN (CRP) AS A BIOMARKER OF INFECTION

Concentrations of C-reactive protein (CRP) were used (mg/L) as the primary PD endpoint in the rabbit CLABSI model. CRP serial levels are useful in the assessment of neonates with acute infection as clinical signs are often unspecific and/or subtle, and blood cultures are only intermittently positive (Benitz et al. 1998). A fall in the value usually assists in the evaluation of infection management (Ehl et al. 1997). CRP quantitative determination constituted a clinically relevant marker of infection to reflect the individual balance between the bacterial burden and the immune system.

2.6.1- CRP determination in the Pre-clinical studies

A two-site enzyme linked immunoassay (ELISA) kit was used to determine CRP measurements in the plasma of rabbits (Caltag Medsystems, UK). Blood samples (0.5 mL) for CRP were taken at 0, 2, 24, 48, 72 and 96 hours post-infection. Serum was stored at -80ºC until analysis.

2.6.2- CRP determination in the Clinical trial

A Multigen CRP Vario® (Abbott, Wiesbaden, Germany) latex immunoassay was used for the immunoturbidimetric determination of CRP in the plasma of patients, implemented in the Abbott Architect ci4100 system. The limit of quantification (LOQ) was < 0.2 mg/L (reported clinically as < 4 mg/L) for the standard and wide range methods (analyte concentration at which the CV=20%). The dynamic range was 0.2-480 mg/L (wide range method) and total precision was ≤ 6%. A CRP cut-off value > 10 mg/L was considered positive. *This method was*

determined in the Pathology Clinical laboratory of Alder Hey NHS Children´s Hospital as standard of care.

2.7- CLINICAL TEICOPLANIN PK STUDY

2.7.1- Study design, study site, patient population and sample collection

Study design

This was a therapeutic clinical Phase IV open-label pharmacokinetic study that collected data acquired from sparse blood sampling strategies to develop a paediatric population pharmacokinetic model of teicoplanin. The aim of the study was to characterize the pharmacokinetic profiles of teicoplanin in paediatric (and neonatal) patients when used in hospital in the routine clinical context.

Study site and patient population

The study was conducted in the Neonatal Unit of the Liverpool Women´s Hospital NHS Foundation Trust (LWFT) and in the selected wards of the Alder Hey Children´s NHS Foundation Trust (AHFT). Paediatric patients (0-<16 years of age) were recruited from Alder Hey Children´s Hospital, which has a catchment of approximately 7.6 million people. In addition, both pre-term and term neonates from 26 to 44 weeks postmenstrual age (PMA) were recruited from Alder Hey Children´s NHS Foundation Trust and Liverpool Women´s NHS Foundation Trust (Liverpool, United Kingdom).

Information on patient-specific covariates which may affect the teicoplanin PK profile and that are routinely gathered during clinical care were captured for the study including: gestational age, post-natal age, concomitant medications, medical history, weight, height and renal function (serum creatinine). In addition, CRP

concentrations were determined as per standard of care and information collected for the study.

Eligibility criteria

All patients that received teicoplanin for proven or suspected methicillin-resistant Gram-positive bacterial sepsis and/or central-line associated infection and likely to survive more than 72 hours were eligible for the study.

Inclusion criteria:

In-patients at Alder Hey Children's NHS Foundation Trust or Liverpool Women's NHS Foundation Trust who are either:

a. Neonates (both term and pre-term (24-39 weeks gestational age), or

b. Infants and Toddlers (aged 1 month to 24 months) or

c. Children aged 2 to <16 years old, and

Who must be receiving teicoplanin for suspected or confirmed Gram-positive bacterial infection while in hospital, as part of their routine clinical care.

Exclusion criteria:

Neonate, Infant/Toddler/Child considered unlikely to survive 72 hours after recruitment (as judged by the clinical team caring for the patient).

The approximate working target number of participants was as follows in each age category:

Age category	**Target number for recruitment**
1. Preterm newborn neonates	12
- 24-27 wks	3
-28-31 wks	3

-32-35 wks -36-39 wks	3 3
2. Term neonates (0-27 days)	12
3. Infants and toddlers (1 month to 23 months)	10
4. Children (2-11 yrs)	10
5. Children (12-<16 yrs)	10

Table 2.1- Working target number of patients to be recruited by age category.

Drug dosage and sample collection

The teicoplanin dosage administration was in accordance with local or national guidelines. Teicoplanin was used at the discretion of the treating neonatologist/paediatrician. The regimen for neonates ≤ 44 weeks PMA was a loading dose of 16 mg/kg, followed 24 hours later by 8 mg/kg administered once daily via a 30- minute i.v. infusion. The regimen for older children (> 1 month old) was the following: three loading doses of 10 mg/kg every 12 hours, followed by 10 mg/kg once daily thereafter via an i.v. bolus administration.

Participants had the usual blood tests as per routine clinical practice. In addition, at the same time as other blood sampling, when possible, an extra aliquot of blood (0.2 ml) was obtained for teicoplanin blood levels. The time of the blood sample was precisely recorded, as with the time, dose and frequency of the teicoplanin, and any other medication that the participant was receiving out.

Sampling strategy

Based, on the available literature on teicoplain pharmacokinetics, our sampling regimen was designed to be: 1, 3, 6 and 24 hours after the dose was infused (over 5 minutes in older children) at first and at steady state dose intervals. An additional wash-out sample was also collected 24 hours after the last dose of

treatment, when possible. The 1 h post dose was determined to estimate the concentration peak or C_{max}, 3 and 6h were determined to estimate the distribution and elimination phases, as well as the 24h trough and wash-out samples to complete the estimation of the terminal elimination (average terminal half-lives described above and others, between 6.5 and 20.5 h in children)(Reed et al. 1997; Tarral et al. 1988; Sánchez et al. 1999; Lemerle et al. 1988; Aarons et al. 1998) The blood was sampled by experienced paediatric/neonatal nurses according to unit policies, which includes measures to minimize pain and distress.

The volume of blood sampled (0.2 mL) complied with the recommendations made by the European Agency Medicines (EMA) and the Medicines for Children Research Network (MCRN) guidelines. Sampling, therefore, did not exceed 3% of the total blood volume during any four-week period or 1% at any single time. If a participant was involved in simultaneous trials, the overall limit of 3% would remain the maximum (Hawcutt et al. 2010). A record of the number of samples taken was kept to assessing the amount of blood taken.

Blood samples were taken either by sampling from an indwelling arterial or venous line (if they were available for blood sampling) or venipuncture or heel prick method. They were taken as per unit policy during the dosing interval as part of a sampling episode required for routine clinical care. Study samples were collected into appropriate bottles and transferred to the laboratory where they were analyzed. The sampling times were calculated from the start of the first infusion of teicoplanin, except for the first sample that was taken 1 hour from the end of the infusion on day 1.

In conclusion, the sampling strategy was therefore as follows: 1 hour from the end of the infusion and 3, 6 and 24 hours from the start of the infusion at each dose

interval. Four samples were taken in the first day of treatment and then at the 5th-7th day of treatment.

Neonates less than 1000 g had only 2 samples taken on alternate times to minimize blood loss but allocated to one of 3 subgroups with 2 specific time points each, to ensure representation of each period. They were assigned to each subgroup by simple random method as they were recruited.

Group	**Weight**		**Sampling times per dosing interval on day 1 and day 5-7 of teicoplanin treatment**			**Maximum blood volume for all study samples*(ml per Kg body weight)**
A	**>1000g**		T1	T3	T24	1.2 ml
	< 1000g	**A1**	T1		T24	0.8 ml
		A2	T1	T3		
		A3		T3	T24	
B	**>1000g**		T1	T6	T24	1.2 ml
	<1000g	**B1**	T1		T24	0.8 ml
		B2	T1	T3		
		B3		T3	T24	

Table 2.2- Sampling strategy subgroups in neonates below and over 1000 grams to minimize blood loss.

Sample time window

To ensure the samples provided accurate data, the samples were required within a 20-minute interval of the allocated sampling time (10 minutes before or after the allocated time). The research team discussed the timing of samples required with the clinical team and parents to ensure care was coordinated at this time and with minimal disruption of patient's routine. However, some additional blood

sampling episodes were required for the purposes of the PK study and this was made clear during the consent process.

Microbiology

Microbiological specimens, including blood culture isolates, were collected as part of routine clinical care and processed in the NHS laboratory. Positive microbiological samples were then stored for MIC investigations, determined by the Epsilon Test (ETest) (bioMérieux, Hampshire, UK) where a manufactured strip containing a predefined concentration gradient of antibiotic is applied to an inoculated plate and following incubation, a clear zone of no growth is produced. Then the MIC was read off the scale printed on the strip following EUCAST/BSAC methodology (Andrews 2006).

2.7.2- Clinical trial methodology and preparation

Feasibility study and planned recruitment time

A prospective feasibility study was conducted during a 4 month-period to evaluate the target recruitment wards in Alder Hey Children´s Hospital. These wards were: PICU, neonatal ward, HDU and Oncology.

In the last 6 years, 50 to 123 neonates had been on teicoplanin each year in the Neonatal Unit at the Liverpool Women's NHS Foundation Trust (minimum and maximum numbers in 2009 and 2007, respectively). That is, a median of 86.5 neonates a year in the 6-year period and about 7 neonates/ month.

Approximately 35-50% of parents will consent to participation in a simple PK study (unpublished data: M. Turner, based on recruitment rate in a previous neonatal ciprofloxacin PK study, which included 4 extra blood tests). That implied

that a number of 2 to 5 neonates could be recruited each month at the same neonatal unit. In order to achieve a target number of 22 neonates (including both pre-term and term), between 5 and 11 months of recruitment was planned to be necessary.

Staff training

Once the recruitment wards were selected, clinical staff was trained on the study characteristics by the clinical investigator (myself) and the lead research nurses (Sarah Siner and Patrick MacGowan). The research team was more specifically trained on the study and standard operating procedures.

Study Procedures

Study Introduction

Both the study and the study team (chief investigator, clinical investigator and research nurses) were introduced verbally to the parents of the child (and to the older child) by a member of the clinical and/or research team.

Informed Consent

Informed consent was obtained by the Chief Investigator, Clinical Investigator and/or nominated deputy as recorded on the Sponsor's Delegation of Responsibilities Log. All individuals taking informed consent had received training in Good Clinical Practice (GCP) and study-specific training.

Consent to enter the study was obtained after a full account had been provided of its nature, purpose, risks, burdens and potential benefits, and the parent(s) has had the opportunity to deliberate. In the case of unmarried parents, the father was only able to provide consent if he was named on the child's birth certificate.

Periods shorter than 24 hours were permitted if the parent(s) felt that further deliberation would not lead to a change in their decision, and provided the person

seeking consent was satisfied that the parent(s) had fully understood and deliberated on the information given. Likewise, periods longer than 24 hours were permitted should the parent(s) request this. The investigator or designee explained that the parent(s) were under no obligation to enter the trial and that they could withdraw their child at any time during the trial without having to give a reason.

A copy of the signed Informed Consent Form (ICF) along with a copy of the most recent approved Parent/Patient Information Sheet (PIS) were given to the study participant. The original signed consent form was kept at the study site, one filed in the medical notes and one file in the trial master file (TMF). A copy of the consent form was also given to the parents.

The study team adopted a process of "ongoing consent" during which the progress of participants and the study were discussed, parents were given repeated opportunities to ask questions, clarify any issues about the study and confirm or withdraw their agreement to their child participating in the study. The aim of this process was to optimize the comfort of parents with the study when the time came for blood sampling or to give parents the opportunity to withdraw consent if they felt that was appropriate.

Discontinuation/withdrawal of participants and stopping rules

Participants had to be withdrawn if the inclusion criteria were no longer met, or if the exclusion criteria were met. Reason for withdrawal was recorded. In accordance with Good Clinical Practice (GCP), parents or legal guardians could withdraw their child or infant from the trial at any time without explanation. If participants exited the study prior to the first study teicoplanin level being obtained, they were not evaluable and therefore were replaced.

Subject/Patient recruitment process

Patient recruitment at a site was only started once the trial team had ensured that the following essential documents were in place:

1. The main REC approval
2. Final sponsorship and host site approval
3. Sponsor agrees that the investigator and the study team have read and understand study-associated responsibilities and the Delegation of Duties Agreement is signed.
4. Study team and clinical team at the site are trained in the study procedures.
5. Signed Clinical Trial Site Agreement (CTSA)
6. MHRA approval for a Clinical Trial of Investigational Medicinal Product (CTIMP)
7. R&D approval

All subjects who wished entering the study were fully screened and their parent(s) consented by the Principal Investigator and/or a nominated deputy as recorded on the Sponsor´s Delegation of Responsibilities Log. Parents were informed about the study using parent information sheets.

Children under 8 yrs, 8-12 yrs and 12-16 yrs were provided with specific age-group patient information leaflets for their assent to participate in the study. However, parent/legal representative informed consent was always sought in children under 16 years old (see annexes).

Study assessments

Screening assessment

Participants were screened to ensure that all of the inclusion criteria and none of the exclusion criteria were met.

Baseline assessment

A clinical assessment consisted of a standard physical examination conducted by a health care professional with appropriate clinical training and experience, and study-specific training, which was in the delegation log.

Demographic information, birth weight, current weight, current height, medical history (including level of care), concomitant medication, details of teicoplanin dosing, microbiology samples and results from current episode (including MIC of the microorganism cultured if available). Blood results from haematology (Hb, haematocrit, full blood count), biochemistry (renal function, albumin, CRP) and blood gas analysis that were required for clinical care were also collected.

Subsequent assessment

Medical history (since baseline), concomitant medication, details of teicoplanin dosing, microbiological samples sent and results (since baseline), teicoplanin serum levels, haematology and biochemistry results.

End of study assessment

A scavenged sample, if possible, was collected 24 hours after completion of teicoplanin study. Clinical data were collected at the end of the study and if required by the clinical team, further haematology and biochemistry results were also collected.

Summary Chart of Study assessments

Study procedure	Following admission to the ward/ unit	At time judged suitable by clinical staff	Clinical suspicion of infection/ sepsis	Clinical decision to start teicoplanin	Sampling Day 1 and day 5-7	Final dose of teicoplanin	24 hours after completion of treatment (only if possible)
Introduction of the study to parents or legal representatives	X						
Consent		X	X	X			
Blood culture as per clinical practice			X				
Gram positive suspected or proven				X			
Enrolment (if eligible)				X			
Teicoplanin administered					X		
Baseline assessment				X			
PK blood sampling ± DNA sampling					X	X	X
Subsequent assessment					X	X	
End of study assessment						X	X

Table 2.3- Summary chart of PK study assessments.

Data management

Confidentiality

All data were handled in accordance with the Data Protection Act 1998. The Case Report Forms (CRFs) did not bear the subject's name or other personal

identifiable data. The subject's initials, Date of Birth (DOB) and trial Identification Number (ID) were used for identification.

Data Collection Tool

Case Report Forms were designed by myself (clinical investigator) and the final version was approved by the Sponsor. All data were entered legibly in black ink with a ball-point pen. It was the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Delegation of Responsibilities Log identified all trial personnel responsible for data collection, entry, handling and managing the database.

For the purpose of this trial data was recorded in the CRF from the source documents, which included medical notes, drug prescription charts and computerised laboratory reporting systems. Then, data were transferred into an electronic CRF (eCRF) and subsequently, to a spread-sheet (e.g Excel®) for further analysis with the appropriate software.

Data handling for analysis

For the pharmacokinetic elements of the study, participant teicoplanin levels were entered into a secure Excel database by Kevin Padmore and transferred to the eCRF by the clinical investigator.

Mathematical PKPD modelling and statistical analysis

Primary endpoints

The primary endpoints were the population PK parameters of teicoplanin drug clearance and volume of distribution for the paediatric, neonatal and joint

population models generated in Pmetrics (version 1.2.6. University of Southern California, California, United States). In addition, when PD information was available and linked to the PK data, population PD parameters (i.e EC50: teicoplanin concentration needed to provide a half-maximal effect in CRP reduction) were also estimated.

Secondary endpoints

The secondary endpoint was the proportion of patients reaching currently recommended drug exposure targets (i.e C_{min} > 10-15 mg/L by day 3-5 of therapy)(The Electronic Medicines Compendium 2014). Additional calculation of drug exposures achieved in terms of area under the concentration-time curve (AUC_{0-24}) was conducted.

Sample size calculation

The exploratory nature means that distributions in the final model are not known in advance. In general, sample size should be based on the number of potential covariate relationships to be studied. Typically, a standard population PK study would include at least 50 patients. Tam and colleagues investigated the sample size necessary to provide robust PK predictions when using population modelling with Monte Carlo simulations to predict PK variability of antimicrobials. In order to obtain reasonably robust predictions, they have recommended that a non-parametric model derived from a sample population size of at least 50 is necessary (Tam et al. 2006).

Mathematical PK/PD modelling

All teicoplanin data were analysed using a non-parametric population modelling methodology (Non-Parametric Adaptative Grid, NPAG) with the population pharmacokinetic software program Pmetrics (version 1.2.6. University of Southern California, California, United States, [http://www.lapk.org/pmetrics.php]) (Neely et al. 2012) for R (version 3.1.0, Institute for Statistics and Mathematics, Vienna, Austria, [http://www.r-project.org/](R Team 2013).

Different structural PK models were evaluated. The inverse of the assay variance was used as the weighting function for all models. The effect of different clinical covariates was assessed during the model-building process, to determine which covariates should be retained in the final model. The final PK model was used to generate empirical Bayesian estimates of population and individual PK parameters for teicoplanin. Given the anticipated PK variability in the population, the Bayesian posterior estimates for each patient's PK parameters from the final PK model were fixed and the PD parameters were then estimated by fitting the PD component of the model to each patient's CRP data. The Bayesian posterior estimates for each subject were used to estimate the concentration–time profiles for teicoplanin and CRP for each patient. Average AUC and trough (C_{min}) for each 24 h of therapy were calculated from the Bayesian posterior estimates.

Summary statistics from the demographic data were analysed with SPSS Statistics version 21 (IBM Corporation, New York, United States).

More detailed information with respect to the clinical and pre-clinical PK/PD modelling methods can be found in Methods section 2.8 and in the specific methods sections of each Results Chapters.

2.7.3- Ethical approval and trial registration

Risk/benefit analysis

The study participants received teicoplanin as part of their routine clinical care at the current local or nationally recommended treatment dose, dose interval, infusion rate and route of administration. Their routine clinical care was not be affected by participation in the study. The majority of additional study samples were obtained at the same time as blood sampling for routine clinical care. This required a small amount of extra blood. If insufficient blood was obtained, analysis for clinical care always took priority. Participants were also asked to contribute extra blood sampling episodes. The additional aliquots or samples were clearly labelled as research samples, batched and analyzed retrospectively. The teicoplanin levels measured in the study were not used in the clinical management of the participants. All sampling episodes were conducted by experienced paediatric or neonatal staff that took account of the needs and preferences of the child and their family. Pharmacological and non-pharmacological analgesia was used in accord with local guidelines and the wishes of participants and their families. In general, participants were not at increased risk of adverse events as there were no additional interventions compared to their routine NHS care. The participants who contributed study specific sampling episodes were exposed only to the minimal risks of sampling in a dedicated paediatric facility.

The benefits were that precise PK models and PK information would improve the care of children treated with teicoplanin in the future.

Management of potential study risks

In order to prevent participants becoming anaemic from excessive additional sampling, the volume of additional blood taken per participant complied with the EMA and MCRN recommendations on paediatric and neonatal research (Hawcutt et al. 2010).

Ethical approval and trial registration

The study was approved by the Medicines and Healthcare Products Regulatory Agency (clinical trial authorisation reference number: 21362/0003/001-0002) and the National Research Ethics Service and Regional Committee (REC: 13/NW/0023). Written informed consent was obtained from parents and/or legal guardians. The study was registered in the European Clinical Trials Database Registry (EudraCT): 2012-005738-12.

2.7.4- Monitoring plan

Study safety monitoring

During teicoplanin therapy, the study team conducted a systematic daily review of patient records until 3 days after the last dose using electronic patient data system and consulted carers and parents in order to identify adverse events.

Clinical blood samples

All Biochemistry/ Haematology analyses carried out during the course of teicoplanin for routine care were assessed for safety parameters. No study-specific blood sampling episodes were undertaken for safety purposes.

Study data precision monitoring

Reliability and data accuracy was monitored according to specific trial tools for the precision of sampling and also to avoid data recording imprecisions.

An internal trial data-monitoring group, led by myself, supervised and ensured the completeness of these procedures.

Quality Control and Quality Assurance

The study was also monitored by the LWFT and the AHFT Research and Development departments.

2.7.5- Regulatory Compliance

Safety reporting

Although the study design reflected the need for information gathered at Phase II of drug development, this was in fact a post-marketing study (Phase IV). The medication is used widely in this age group. The expected adverse events are described in the summary of product characteristics (SmPC). The aim of the study was not to report on safety. The Sponsor did not wish to receive reports about well-recognised adverse events during teicoplanin administration. All health care professionals were encouraged by the MHRA to report all suspected adverse drug reactions to the MHRA using the Yellow Card system. This study did not seek to duplicate that reporting. Accordingly, the Sponsor defined a list of expected adverse events it did not wish to be reported. The trial included mechanisms for the expedited reporting of suspected, unexpected serious adverse reactions.

2.8- PK/PD MATHEMATICAL MODELLING

2.8.1- Population PK/PD modelling

Data from the PK and PK/PD studies were analysed using a population non-parametric methodology with Pmetrics. This software is an R library for

pharmacokinetic/dynamic modeling which includes the population program Non-Parametric Adaptive Grid Algorithm (NPAG) (Neely et al. 2012). In NPAG, the ordinary differential equations defining the structural pharmacokinetic (and/or pharmacodynamic models) are solved on a multidimensional "box" of pre-specified size, which is first sampled using a low-discrepancy Faure generator to create an initial grid of discrete support points. Each support point is a vector consisting of one value for each dimension of the box. Each value corresponds to a PK or PD parameter. The corresponding likelihood for each combination of data and grid-point is computed to produce a likelihood matrix. The number of support points contained within the grid is reduced via the exclusion of low probability grid points. Each parameter (dimension) of the remaining points is perturbed a fixed percentage of the pre-specified parameter range, while simultaneously fixing the other parameter values. These new grid points are added (in effect new daughter grid points are "sprinkled" around the initial grid point) and the likelihood value for the expanded grid is calculated. There is an iterative reduce-expand-reduce cycle using decreasing perturbations until convergence is reached (i.e. the log-likelihood value of the latest grid does not increase over that which immediately precedes it). At every step, the grid likelihood is calculated using a highly efficient convex program. The final distribution (reduced grid and associated likelihoods) is saved. In order to ensure that a local minimum has not been found, the entire optimization is repeated until the likelihood of the final grid does not change over two successive optimizations. The NPAG algorithm makes no assumption regarding the shape of the population distribution except that the optimal distribution is discrete and of size no greater than the number of observed patients. One reason that NPAG is fast is that size of the

likelihood matrix is reduced to approximately the number of patient observations very early during the optimization.

One distinct advantage of using a population methodology is the ability to obtain: (1) estimates of measures of both central tendency and dispersions for the population as a whole; and (2) estimate the pharmacokinetic parameters for each individual within the population. This is achieved using Bayesian estimation, by defining the *maximum a posteriori* probability in the following manner:

$$Objective\ function = \sum_{j=1}^{m}\left(\frac{P_{patient} - P_{population}}{Standard\ Deviation_{population}}\right)$$

where: P is the parameter value in question. The objective function provides a measure of the difference between the model prediction and the observed data and is minimized in the optimization process. The population mean, median or modal values can be used as the Bayesian prior in order to obtain the Bayesian estimates for the individual subjects—the choice depends on the fit of the observed-versus-predicted values and associated measures of precision and bias.

These measures to assess whether a model is well fitted to the data include, but are not limited to:

a. Observed-versus-predicted values before and after the Bayesian step
b. Model parameter values
c. AIC (Akaike information criterion), BIC (Bayesian Information Criterion)
d. Measures of precision and bias
e. Simulated model behaviour: e.g Visual Predictive Checks (VPCs)

Pmetrics enables weighting by 1/y, where y is usually the standard deviation of the observation. For pharmacokinetic data the most orthodox approach is to use information from the assay variance. Pmetrics enables a polynomial to be used as the weighting function in the following way:

Standard Deviation=C0+C1*concentration+C2*concentration+ C3*concentration.

This relationship is estimated by fitting a polynomial of the appropriate order (e.g. in excel) to the relationship between concentration (the independent variable) and the observed standard deviation across various parts of the assay (e.g. low, medium and high concentrations).

Error polynomials for bacterial density counts (log_{10} CFU/mL) and CRP measurements were obtained by fitting the same structural mathematical model to the pharmacokinetic and pharmacodynamic data from each of the infection models, using the maximum likelihood estimator in the parametric program ADAPT 5 (W. X. D'Argenio DZ, Schumitzky A 2009).

2.8.2- Monte Carlo Simulations and PTA analysis

Monte Carlo simulations were used to explore the extent of PK variability in a larger simulated population and its influence on the achievement of target drug exposures and PD endpoints. In this thesis project two different programs and methodologies were employed.

Firstly, ADAPT 5, which uses a parametric expectation maximization method (W. X. D'Argenio DZ, Schumitzky A 2009). Using this method, the structural mathematical model (a series of ordinary differential equations), a mean estimate of each of the PK model parameters and the variance of these parameters must be known. A diagonal covariance matrix may be entered which involves the variance of

each parameter vector. Alternatively, the full covariance matrix may be entered, which also describes any relationship between the parameters. Either normal or log-normal distributions for each of the parameters are assumed. The ability to recapitulate the original parameter values and their dispersions was used to select which parameter distribution was utilised.

Secondly, Pmetrics was used, which uses a semi-parametric sampling methodology, rather than assuming a normal or a log-normal distribution (Neely et al. 2012). The final structural PK model was used, which consisted of a number of support points. Each support point comprised a set of model parameter values and the associated probability of those values that describe groups of patients in the population. Each support point served as the mean for a multivariate normal distribution, weighted by the probability of the point, with covariance equal to the covariance matrix of the full model divided by the number of points. This semi-parametric sampling from this weighted, multivariate, multi-modal normal distribution, generated a novel population of 1000-5000 parameter sets for 1000 to 5000 patient profiles receiving a teicoplanin regimen.

Finally, Monte Carlo simulations were also used to study the probability of target attainment (PTA) in the simulated populations of standard and candidate teicoplanin dosage regimens. This analysis allowed: 1) the bridging of experimental findings with respect to relevant PD targets to human patients and 2) to reflect on the adequacy of current EUCAST *in vitro* susceptibility breakpoints (Turnidge & Paterson 2007). The distribution of the MRSA MICs determined using EUCAST methodology from 0.032 to 16 mg/L were plotted against the probability of success to achieve a predefined target exposure (i.e. AUC:MIC ratio). Fractional target attainment was calculated for the various regimens and the AUC/MIC targets

against the MIC distribution for MRSA and teicoplanin (European Committee on Antimicrobial Susceptibility Testing 2010).

2.8.3- Exposure-effect relationships

To determine the PK/PD index that best described bacterial killing, the suppression of drug resistance (in pre-clinical models) or CRP reduction *in vivo* and in patients, scatter plots were constructed that related the AUC:MIC, C_{max}:MIC and C_{min}:MIC with both the observed antibacterial effect (or CRP reduction) and the emergence of a drug resistant subpopulation from the pre-clinical models. A non-linear regression Emax inhibitory model was fitted to the data using Prism software for Mac OSX (GraphPad Software Inc., La Jolla, CA). The coefficient of determination and visual examination of the fit was used to discriminate the various pharmacodynamic indices.

2.8.4 Dose optimisation software

The dose optimisation software (BestDose) is based on the concepts developed by the University of Southern California Laboratory for Applied Pharmacokinetics and Bioinformatics (LAPKB) (http://www.lapk.org) which uses a multiple-model Bayesian adaptive control algorithm. A multiple-model approach allows the use of multiple discrete individual models (at most, one for each patient) as a discrete collection of points to find the most likely population parameter distribution (Macdonald et al. 2008). This software was used in the last RESULTS chapter of the thesis (chapter 7) to: 1) update the general paediatric population teicoplanin´s PK parameter probability distribution for an individual patient, and 2)

to use the updated, individual model to match measured concentrations, predict future concentrations, and calculate dosages to achieve target concentrations.

The teicoplanin BestDose software included the structural final model equations relating input (dosing information) to output (plasma concentrations) and the discrete joint probability distribution of the values of the equation variables (PK parameters) in the population, consisting of a discrete number of support points and their associated probability (the Bayesian prior). It also included a patient "past" file that contained the observed drug concentrations and details of the administered teicoplanin regimen, and a patient "future" file, which contained the target drug concentrations deemed to be optimal for the patient, as well as the initial estimates of the required drug dosages and frequency of administration. The software then calculated the drug regimen having the least weighted squared error in target goal achievement over the Bayesian posterior distribution.

Detailed methodology information with respect to the PK/PD modelling can be found in specific methods section of the Results Chapters.

Chapter 3

Pharmacokinetics and Pharmacodynamics of Vancomycin for Coagulase-Negative Staphylococcal infection: Experimental Basis for Optimal Use of Vancomycin in Neonates

3.1- ABSTRACT

Objectives: Coagulase-negative staphylococci (CoNS) are the most common cause of neonatal late onset sepsis. Information on the vancomycin PK/PD against CoNS is limited. The study aim was to characterize the vancomycin PK/PD relationships for CoNS and investigate neonatal optimal dosage regimens.

Methods: A hollow fibre and a novel rabbit model of neonatal central-line associated bloodstream CoNS infections were developed. The results were then bridged to neonates by use of population pharmacokinetic techniques and Monte Carlo simulations.

Results: There was a dose-dependent reduction in the total bacterial population and the C-reactive protein levels. The AUC:MIC and C_{max}:MIC ratios were strongly linked with total and mutant resistant cell kill. Maximal amplification of resistance was observed *in vitro* at *f*AUC:MIC of 200 mg*h/L. Simulations predicted that neonates < 29 weeks postmenstrual age are under-dosed with standard regimens.

Conclusions: The AUC:MIC and C_{max}:MIC ratios are the pharmacodynamic indices that best explain total and resistance cell kill in CoNS infection. This suggests that less fractionated regimens are appropriate for clinical use and continuous infusions may be associated with increased risk of emergence of antimicrobial resistance. This study has provided the pharmacodynamic evidence to inform an optimized neonatal dosage regimen to take into a randomized controlled-trial.

3.2- INTRODUCTION

Neonatal sepsis is associated with poor neurodevelopmental outcomes (Adams-Chapman & Stoll 2006; Mitha et al. 2013). This occurs independently of the causative pathogen, probably as a result of the systemic inflammatory response. Late onset sepsis (LOS) in neonates is an important cause of global morbidity, prolonged hospital stay and mortality (Levit et al. 2014; Stoll et al. 2010; Hornik et al. 2012). CoNS account for 30-54% of all cases of LOS (Vergnano et al. 2011; Stoll et al. 1996; Stoll et al. 2002) and over 80% of CoNS are methicillin-resistant (Hilmar Wisplinghoff et al. 2003). Central line-associated bloodstream infections (CLABSIs) are the most common risk factor of CoNS sepsis in neonates (Milstone et al. 2013). However, the treatment of these infections in neonates is problematic. Line removal is often not possible because of clinical instability and/or difficulty re-establishing venous access (Karlowicz et al. 2002b). In contrast, in adults, line infections caused by CoNS are usually readily treatable with glycopeptides and line removal. There are few, if any, clinical sequelae in the absence of other indwelling foreign devices or material.

Vancomycin is a first-line agent for the treatment of serious infections caused by resistant Gram positive pathogens (Mermel et al. 2009) and is increasingly used in the neonatal intensive care unit (NICU)(Clark et al. 2006). Reduced susceptibility of CoNS to vancomycin has been reported (Schwalbe et al. 1987; Schwalbe et al. 1990; Sanyal et al. 1993b; D. Sanyal & Greenwood 1993; Sanyal et al. 1991; Raad et al. 1998; Srinivasan et al. 2002; Rasigade et al. 2012; Hira et al. 2013). There is surprisingly little information to guide the optimal use of vancomycin in neonates. Hence, it is important to find ways to optimally treat CoNS in neonates to eradicate the organisms and minimise inflammation.

In this chapter, we defined the PK/PD of vancomycin for CoNS infections using both a hollow fibre infection model (HFIM) and a novel rabbit model of neonatal CLABSI. *S. epidermidis* and *S. capitis* were used as the challenge strains. We considered the antibacterial effect of vancomycin, the emergence of drug resistance and reductions in circulating concentrations of C-reactive protein (CRP) as pharmacodynamic endpoints. We bridged these experimental results to neonates to identify regimens for further study in clinical trials.

3.3- MATERIALS AND METHODS

3.3.1- Organisms, Susceptibility Studies and Mutational Frequency

Coagulase-negative staphylococci (CoNS) were used for all experiments. Five clinical strains were recovered from two different NICUs in the United Kingdom: three strains of *S. epidermidis* (122648, 122761, 121164) and two strains of *S. capitis* (122828, 062012) (courtesy of Dr Neal; Liverpool Women´s Hospital). The MIC for the 5 strains was determined using CLSI and EUCAST microbroth dilution methodologies on 10 separate occasions (Clinical and Laboratory Standards Institute 2012; European Committee on Antimicrobial Susceptibility Testing 2015).

The mutational frequency of a less-susceptible population was calculated as the ratio of the number of colonies that grew on vancomycin-containing Mueller-Hinton (MH) agar plates at a concentration of 4 mg/L divided by the number of colonies that grew on drug-free agar (Nicasio et al. 2012).

3.3.2- Vancomycin concentration determination

Analyses of vancomycin concentrations in rabbit plasma and MH broth were performed on an Ultra High performance Liquid-Chromatography tandem Mass Spectrometry system (UHPLC/MS-MS) as described previously in general methods (chapter 2). *This method and analysis was performed by Joanne Goodwin and Sarah Whalley.*

3.3.3- *In vitro* Model of Neonatal Bloodstream Infection in a Hollow Fibre System

Vancomycin hydrochloride for intravenous infusion (Vancocin 500 mg, Flynn Pharma Limited, Dublin, Ireland) was used. A HFIM was used to simulate a typical neonatal pharmacokinetic profile and study the pharmacodynamic responses of CoNS to different regimens of vancomycin (Felton et al. 2013). An elimination half-life of 13 hours for vancomycin was used (Seay et al. 1994).

For each experiment, fresh bacterial isolates were grown on blood agar plates (Oxoid Limited, Hampshire, UK) and incubated at 37º C for 24 hours. Bacteria were then inoculated into the extra-capillary space of each hollow-fibre (HF) cartridge. The initial inoculum used was approximately 4.5 $\log_{10}$ CFU/mL. This inoculum was expected to reach a density of approximately 9-10 $\log_{10}$ CFU/mL 24 hours post-inoculation and before treatment in the HF. The desired inoculum was confirmed by quantitative culture on MH agar plates (Sigma-Aldrich, Hants, UK). The HFIM was incubated at 37ºC in ambient air. A bacterial density of > 10^2 CFU/mL from a sonicated catheter by quantitative broth culture reflects catheter colonization in patients. For quantitative blood cultures, a colony count of microorganisms grown from blood obtained through a catheter hub should be at least 3-fold greater than the colony count from blood obtained from a peripheral vein to best define a catheter-related bloodstream infection (CRBSI)(Mermel et al. 2009). Despite using the IDSA guidelines for the diagnosis of CRBSI for the choice of inoculum sizes, to our knowledge, there is no reported mean bacterial density data from blood cultures in neonates/children to be diagnostic of LOS or CLABSI.

A total of five experiments each consisting of seven hollow fibre arms (control and six drug-treated arms) were conducted. Each experiment was performed with a

different CoNS strain (three for *S. epidermidis* and two for *S. capitis*).

3.3.4- *In Vitro* Pharmacokinetic and Pharmacodynamic Studies

Initial dose-finding studies were conducted with neonatal-like concentration-time profiles of vancomycin that corresponded to equivalent human dosages of 7.5, 15 and 30 mg/kg/day. The total daily dose of vancomycin was administered as: (a) 1 hour infusion every 24 hours (q24h); (b) fractionated as half the total daily dose administered twice daily as 1 hour infusion (q12h) or (c) via a 24 h continuous infusion (CI). Treatment was initiated 24 hours post-inoculation. PK samples were collected at 0, 1, 2, 6, ±12- if q12-hourly administration- and 24 h post-end of infusion (1 h infusion) at first dosing interval and at steady-state (day 6 of therapy). Experiments were conducted for up to 10 days to simulate the typical duration of clinical therapy and enable the generation of antimicrobial resistance. The AUC, C_{max} or Peak and C_{min} vancomycin concentrations at steady state and the burden of bacteria at the end of therapy were determined.

Total and vancomycin resistant bacterial density were quantified at each time-point by plating on drug-free and vancomycin (4 mg/L-2xMIC and clinical breakpoint-) containing MH agar plates (Sigma-Aldrich)(European Committee on Antimicrobial Susceptibility Testing 2015).

Bacterial samples (1 mL) were withdrawn from each of the HF cartridges and aliquots of 0.1 mL were plated on agar plates at 0, 2, 6 and 24 hours post-infection and every 24 hours thereafter immediately prior to dosing. “Resistant” bacteria were defined as the number of colonies counted on the drug-containing plates.

3.3.5- *In vivo* Model of Neonatal CLABSI in Rabbits

All experiments were conducted under Home Office project license (40/3630) and approved by the University of Liverpool Animal Welfare Committee. A non-neutropenic rabbit model of neonatal CLABSI was developed and used to study the response of CoNS to different regimens of vancomycin (Vancocin 500 mg, Flynn Pharma Limited). Male New Zealand white rabbits (2.68-3.67 kg) were used for all experiments. Two clinical strains of CoNS (*S. epidermidis* 122648 and *S. capitis* 122828) obtained from neonates were used. Forty-two rabbits were studied (22 rabbits were infected with *S. epidermidis* and 20 rabbits with *S. capitis*). Each experiment consisted of 6 rabbits (2 controls and 2 different dosage groups of two rabbits each).

A 1 mL volume of 8 $\log_{10}$ CFU/ml was administered via the central catheter of each rabbit and locked with 0.5 mL of lock solution (500 IU/mL of heparin in 10% dextrose). After two hours up to 0.5 mL of lock solution was removed and the line flushed with 0.5 mL of sterile 0.9% saline. This inoculum was designed to establish a non-lethal model of CLABSI in the rabbits. Experiments lasted for 96 hours. All rabbits were sacrificed 0.5h after the final samples on day 4. At autopsy, the catheters were removed and the tip taken for quantitative culture.

3.3.6- Pharmacokinetic and Pharmacodynamics Studies In a Rabbit Model of CLABSI

Vancomycin therapy was initiated 24 hours post-inoculation and was administered i.v. via the marginal ear vein. Dosages of 10, 20, 50, 100 and 150 mg/kg/day were administered once and/or twice daily, as two divided doses, via an i.v. bolus based on previous dose-finding studies (Nicolau et al. 1993; Ahmed et al. 1999). Blood (0.5 mL) samples for pharmacokinetic analysis were collected from each rabbit

during the first dosing interval and then at steady state. Samples were collected pre-dose and at 2, 4, 12 and 24 hours post-dose from the opposite marginal vein to the vein used for the administration of the drug. Plasma samples were stored at -80ºC until analysis.

Concentrations of CRP were used (mg/L) as the primary pharmacodynamic endpoint in rabbits and determined using a commercially available ELISA kit (Caltag Medsystems, Buckingham, UK). Blood samples (0.5 mL) for CRP were taken at 0, 2, 24, 48, 72 and 96 hours post-infection. Serum was stored at -80ºC until analysis.

Blood samples (0.5 mL) were cultured in paediatric culture media bottles (BacT/Alert® FA plus, Biomérieux SA, Lyon, France) before the first dose and every 24 hours thereafter, and incubated at 37ºC for 48 h before plating 0.1 mL of blood culture medium on drug-free and drug-containing MH agar plates (i.e. vancomycin at 4 and 8 mg/L) (Sigma-Aldrich).

Quantitative counts from the tip of the catheter that was removed at autopsy were estimated. The catheter tips were inserted in 5 mL of PBS and sonicated for 15 minutes (60°C) before plating 0.1 mL of serial 10-fold dilutions on drug-free and drug-containing MH agar plates (4 and 8 mg/L) (Sigma-Aldrich).

3.3.7- PK/PD Mathematical Modelling

All PK and PD data were co-modelled using a non-parametric population methodology using Pmetrics v.1.2.9 (Neely et al. 2012). The structure of the PK/PD mathematical model fitted to HFIM PK/PD data was based on a previously published model of bacterial resistance, (Gumbo et al. 2004) and took the following form:

$$(1)\ \frac{dX1}{dt} = R(1) - \left(\frac{CL}{Vc}\right) * X1$$

(2) $\frac{dx2}{dt} = KgmaxS * X2 * \left(1 - \frac{(X2+X3)}{POPmax}\right) - \left(\frac{KkmaxS*x2*\left(\frac{x1}{Vc}\right)^{HS}}{(C50S^{HS}+\left(\frac{x1}{Vc}\right)^{HS}}\right)$

(3) $\frac{dx3}{dt} = KgmaxR * X3 * \left(1 - \frac{(X2+X3)}{POPmax}\right) - \left(\frac{KkmaxR*x3*\left(\frac{x1}{Vc}\right)^{HR}}{(C50R^{HR}+\left(\frac{x1}{Vc}\right)^{HR}}\right)$

Equation 1 describes the rate of change of the amount of vancomycin (mg) in the central compartment (X1). **Equation 2** and **3** describe the rate of change of burden of a susceptible bacterial population (S) and a resistant/mutant (R) bacterial population in the HFIM. (POPmax (CFU/mL): the theoretical maximum bacterial density; KgmaxS/R ($\log_{10}$ CFU/mL/h): maximum rate of growth in both populations; KkmaxS/R ($\log_{10}$ CFU/mL/h), the rate of bacterial killing induced by vancomycin; C50S/R (mg/L): vancomcyin drug concentrations that produce half-maximal killing; HS/HR: slope functions for killing).

The model fitted to the rabbit CLABSI PK/PD data was similar, but incorporated an additional term to describe immunological killing because a decline in CRP levels was also seen in control rabbits that only received vehicle.

(4) $\frac{dX1}{dt} = R(1) - \left(\frac{CL}{Vc}\right) * X1 - kcp * x1 + kpc * X2$

(5) $\frac{dX2}{dt} = kcp * X1 - kpc * X2$

(6) $\frac{dX3}{dt} = (Kgmax * X3 * (1 - \left(\frac{X3}{POPmax}\right) - (Kkim * X3 * X4) - \left(\frac{Kkmax*X3*\left(\frac{X1}{Vc}\right)^{H}}{(C50^{H})+\left(\frac{X1}{Vc}\right)^{H}}\right)$

(7) $\frac{dX4}{dt} = Kimax * X4 * \left(1 - \left(\frac{X4}{IMAX}\right)\right)$

Equation 4 and **5** describe the rate of change of vancomycin (mg) into and from a central (X1) and a peripheral compartment (X2). **Equation 6** and **7** describe the rate of change of CRP concentrations (mg/L) (X3) in the rabbits and change of immune

system function (X4) over time. (IMAX, Kimax and KKim: theoretical maximum rise, rate of rise of the immune function and rate of CRP suppression exerted by the immune system, respectively).

The fit of each of the PK/PD models to the respective datasets was assessed in the following ways: (1) the log-likelihood value; and (2) both the coefficient of determination (r^2) of the linear regression and visual inspection of the observed-predicted plots before and after the Bayesian step.

To determine the PK/PD index that best explained bacterial killing and the emergence of drug resistance in the HFIM, scatter plots were constructed that related the AUC:MIC, C_{max}:MIC and C_{min}:MIC with both the observed antibacterial effect and the emergence of a drug resistant subpopulation. A non-linear regression model was then fitted to the data using Prism software (GraphPad Software Inc., La Jolla, CA). The coefficient of determination was used to discriminate the various pharmacodynamic indices.

3.3.8- Neonatal Pharmacokinetic Data and Monte Carlo Simulations

The experimental data were bridged to the clinic using a recently developed population pharmacokinetic model of vancomycin for neonates '(Dr. W. Zhao et al, INSERM, personal communication)'. The model was developed from plasma concentrations obtained from 1463 neonates with post-menstrual age (PMA) range 23.3-52.4 weeks and weight range 415-11400 g, from multiple investigators in the following age groups: (1) < 29 weeks PMA, n= 335; (2) 29-35 weeks PMA, n=618; and (3) >35 weeks PMA, n=510.

Monte Carlo simulations were performed using NONMEM (performed by Dr W. Zhao). The currently recommended vancomycin regimens for each neonatal age group were studied, which are as follows: <29 weeks 15 mg/kg q24h; 29-35 weeks 15 mg/kg q12h and >35 weeks 15 mg/kg q8h (Sharland et al. 2016). The AUC at steady state was determined for the simulated neonates using this regimen. A number of optimized regimens were then studied, with the goal of achieving parity in drug exposure (AUC) across the different age groups.

CRP data from a cohort of 10 neonates (aged 26 weeks-39 weeks PMA, weight range 690-5080 g) recruited as part of a teicoplanin pharmacokinetic study (EudraCT number 2012-005738-12) was used to place the rabbit CRP values profile in a clinical context.

A range of drug exposures (AUCs) linked to the predicted CRPs at 96 h (last experimental time-point) was established by means of individual population simulations conducted in ADAPT 5 (X. W. D'Argenio DZ, Schumitzky A 2009) using the population PK/PD parameter medians from the rabbit PK/PD mathematical model. The number of simulated neonates achieving the range of drug exposures was then matched to the predicted linked population CRPs for both standard and optimized therapies. Measures of central tendency and dispersion for the CRP levels were calculated.

3.4- RESULTS

3.4.1- Strains, MICs and mutational frequency

The broth microdilution modal MIC for all the strains by both methodologies used was 2 mg/L. All the strains were oxacillin resistant by Etest (Oxoid). Following 48-h incubation, the frequency of mutants able to grow on plates that contained 2xMIC was between $1.7x10^{-3}$ (*S. capitis* 122828) and $8.19x10^{-6}$ (*S. epidermidis* 121164).

3.4.2- HFIM of CoNS

A human neonate-like vancomycin half-life and plasma concentration-time profiles were readily generated in the HFIM (**Figure 3.1**). The different species and strains of CoNS grew well in the HFIM. The initial density of organisms was approximately 4.5 $\log_{10}$ CFU/mL, which grew over 24 hours to a final density of approximately 10 $\log_{10}$ CFU/mL.

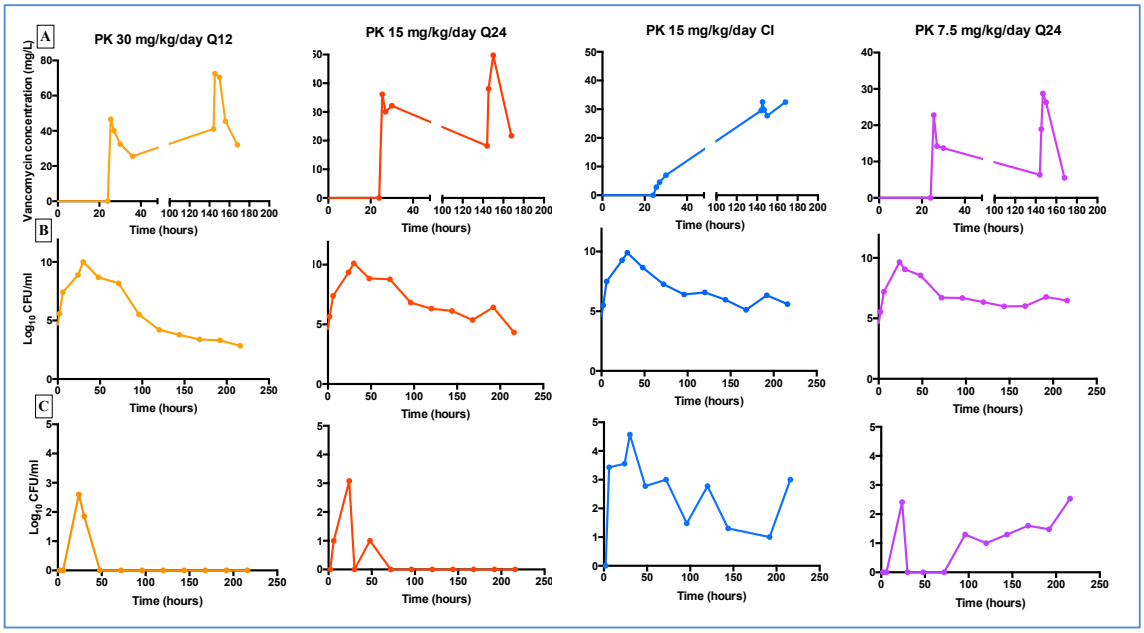


Figure 3.1- Pharmacokinetics and pharmacodynamics of vancomycin against *S. epidermidis* 122648 (raw data) to show: A) The PK profiles of 30, 15 and 7.5 mg/kg/day, B) Total population

bacterial density decline (log_{10} CFU/mL) and C) Resistant sub-population bacterial density decline (log_{10} CFU/mL).

3.4.3- Pharmacodynamics of Vancomycin against CoNS in the HFIM: Dose Finding Studies

There was a dose-dependent decline in the total bacterial density with increasing drug exposure with all the strains investigated. In contrast, lower dosages of vancomycin (7.5 mg/kg/day) resulted in the emergence of a vancomycin resistant population, which was not observed with the use of higher dosages (30 mg/kg/day) **(figure 3.2.A and B).**

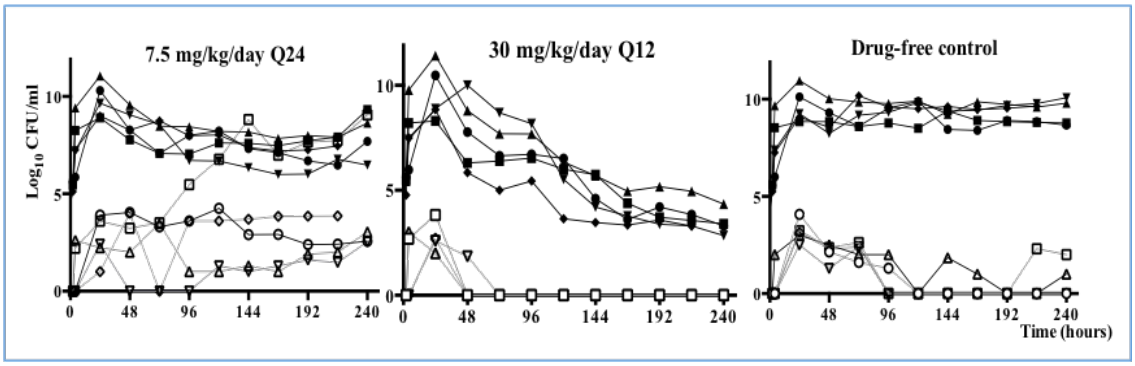


Figure 3.2.A-Pharmacodynamics of vancomycin against the 5 CoNS strains investigated in the HFIM (raw data). The figure shows the bacterial density decline (log_{10} CFU/mL) of the total population (on top, filled symbols) and the resistant sub-population (bottom, empty symbols) for the lower (7.5 mg/kg/day) and higher (30 mg/kg/day) dosages studied plus the drug-free control experiments.

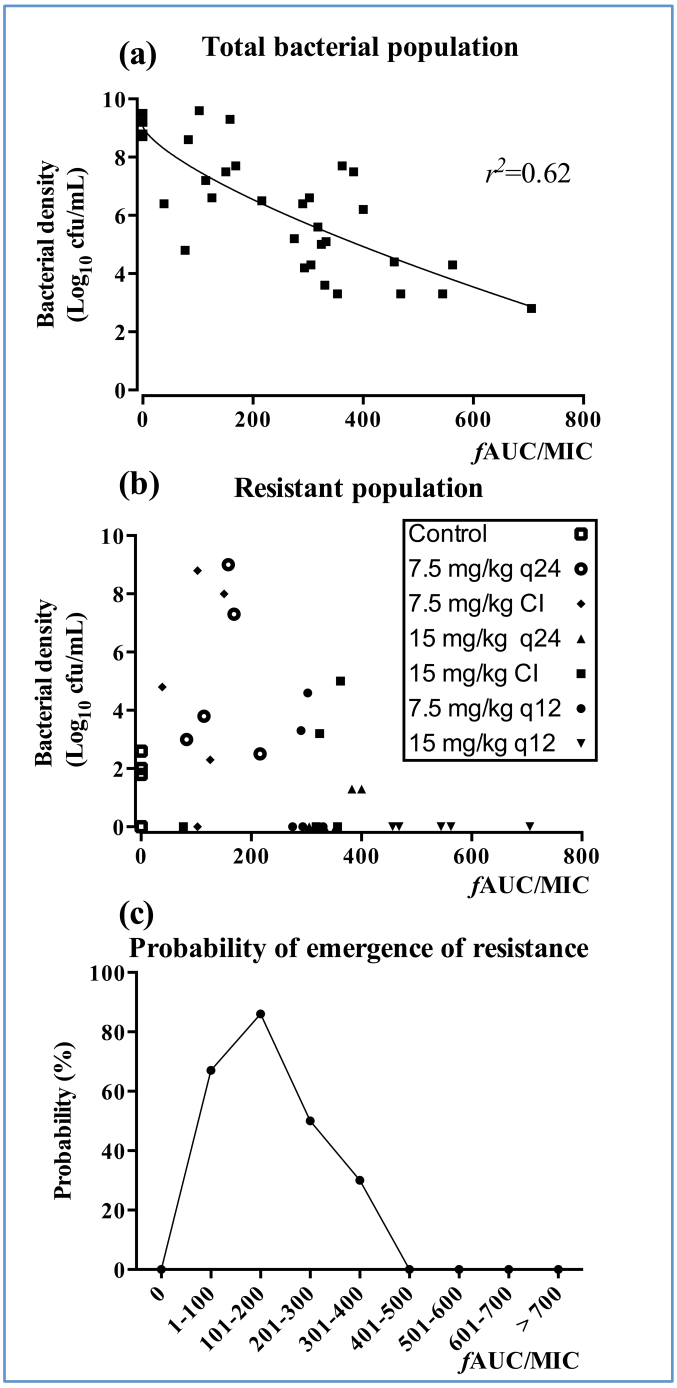


Figure 3.2.B- Pharmacodynamics of vancomycin against five CoNS strains in the HFIM after PK/PD population modelling.

Panel (a) shows the total bacterial density (log_{10} CFU/mL) decline along the different *f*AUC/MIC reached at steady state (96-120 h) in all the experiments. An Emax sigmoid inhibitory model was fitted to the data (r^2 =0.62). Panel (b) shows the resistant bacterial population decline along the same PD index. Panel (c) shows the probability of emergence of resistance compared to controls with each 100 range of *f*AUC/MIC ratios at steady state.

3.4.4- *In vitro* Dose Fractionation Studies

A daily dosage of 15 mg/kg/day resulted in logarithmic killing (> 4 $\log_{10}$ CFU/ml cell kill) regardless of the schedule of administration, but daily administration of drug achieved the greatest logarithmic kill. The emergence of vancomycin resistance was more pronounced in the continuous infusion arms compared with intermittent therapy for strains *S. epidermidis* 121164, *S. capitis* 062012 and *S. capitis* 122828 regardless of the mutational frequency **(Figure 3.3).** Regression analyses, using an inhibitory sigmoid Emax model showed a strong correlation between both fC$_{max}$:MIC and fAUC:MIC and bacterial killing (r^2 = 0.95 for both indices). Nonlinear regression analysis also showed a strong association between fC$_{max}$:MIC and fAUC:MIC and suppression of vancomycin resistance for all the strains, including *S. capitis* 122828, which had the highest mutational frequency of resistance **(Figure 3.4).** %fT> MIC was not investigated because all the dosage regimens fractionated had T> MIC of 100 %.

A vancomycin fAUC/MIC ratio at steady state > 400 mg*h/L was associated with near maximal bacterial killing and suppression of emergence of resistance **(Figure 3.2.B a and b).** Progressively higher drug exposure of vancomycin (AUC:MIC) resulted in progressively higher degrees of bacterial killing. An "inverted U" was observed with maximal amplification of resistance with a fAUC:MIC of approximately 200 **(Figure 3.2.B c).** The PK/PD parameter values from the population PK/PD model are shown in table 3.1.

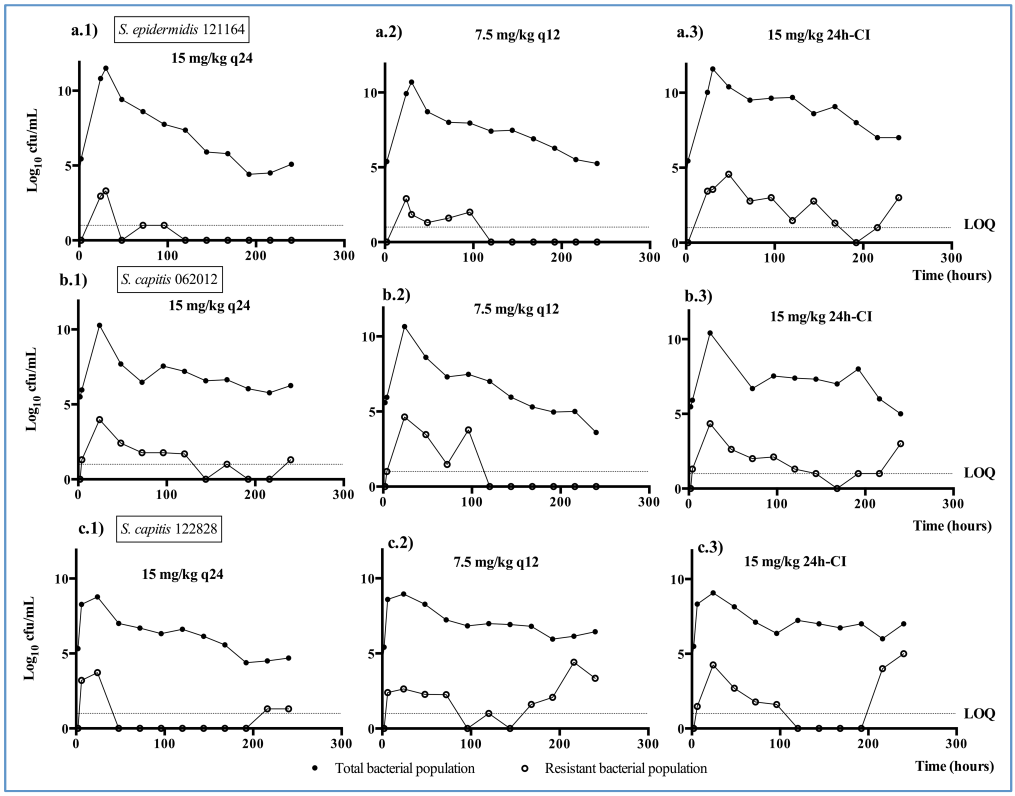


Figure 3.3- Dose-fractionation vancomycin studies (15 mg/kg/day) in the HFIM.

a) *S. epidermidis* 121164, the strain with the lowest mutational frequency to resistance, b) *S. capitis* 062012 and c) *S. capitis* 122828. Regimens consisted of 15mg/kg once daily-q24(1), twice daily-q12 (2) and 24h continuous infusion-CI (3) in the HFIM. Full symbols show the data points (bacterial density count in drug-free plates) for the total bacterial population. Clear symbols represent data points (bacterial density count in 4mg/L vancomycin drug-containing plates) for the resistant sub-population. Dotted lines represent the lower limit of quantification (LOQ) of 1 $\log_{10}$ CFU/mL.

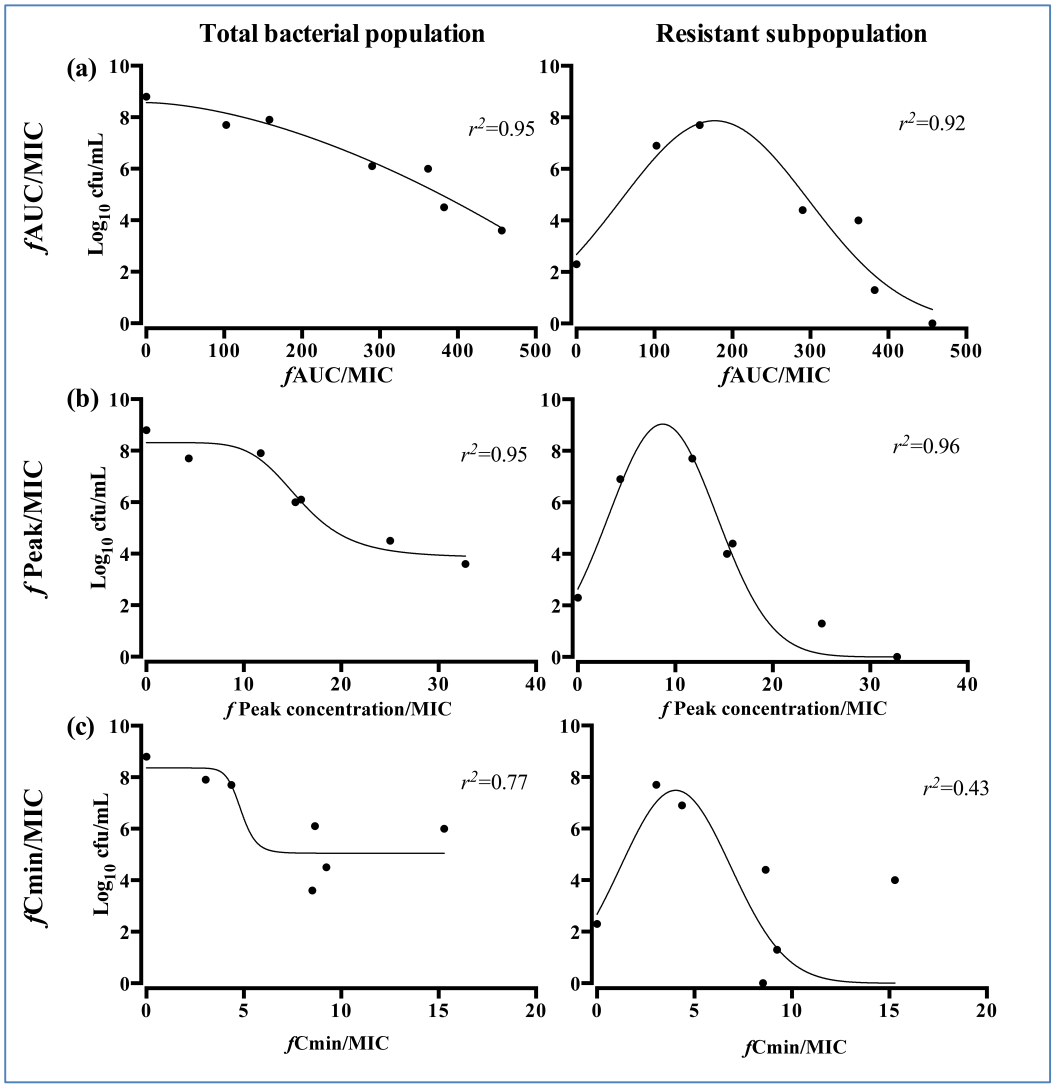


Figure 3.4- Pharmacodynamics of vancomycin against CoNS (*S. capitis* 122828).

The strain with the highest mutational frequency to vancomycin resistance, in the HFIM. The endpoint is the bacterial density count (Log_{10} CFU/mL) at the end of 9 days in the HFIM. An inhibitory sigmoid Emax model was fitted to the total bacterial population versus the PD index and a non-linear regression model was fitted to the resistant subpopulation versus PD index (a): *f*AUC/MIC; (b): fC_{max}/MIC; (c): fC_{min}/MIC).

3.4.5- Vancomycin Pharmacodynamics in the CLABSI Rabbit Model

Vancomycin was well tolerated with regimens ≤ 100 mg/kg/dose. Higher dosages caused acute infusional toxicity that was lethal. Vancomycin induced a dose-dependent decline in CRP **(Figure 3.5 a, b, c).** A neonatal CRP profile during teicoplanin therapy in a cohort of 10 neonates with suspected or confirmed CoNS sepsis was comparable to the CRP profiles observed in rabbits **(Figure 3.5 d).**

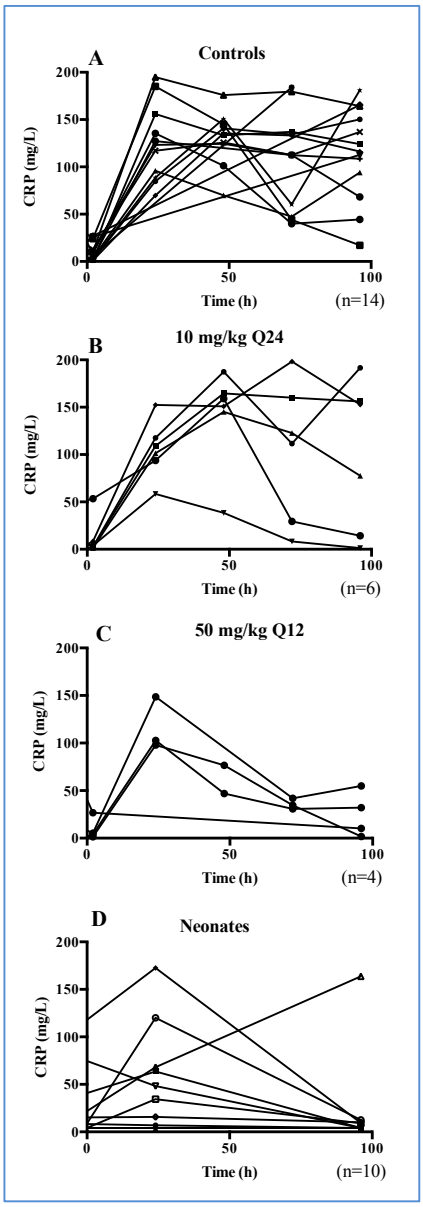


Figure 3.5- Concentration-time profile of CRP in rabbits and neonates.

(a) Control rabbits and for the rabbits receiving the lowest and highest dose of vancomycin along the 4 days of experiment (b) and (c), respectively in the CLABSI rabbit model. Panel (d) shows the CRP profile in neonates at 0, 24 (first day of treatment) and 96 h of therapy with teicoplanin for comparison.

The median estimated clearance in the PK/PD model was 0.576 L/h and the V_c (volume of distribution in the central compartment) was 0.56 L. The distributional and elimination $t_{1/2}$ were 0.18 and 0.9 hours, respectively. The r^2 for the observed-versus-predicted plots obtained after the Bayesian step from the PK-PD population model for vancomycin and CRP concentrations was 0.957 and 0.756, respectively. The PK/PD parameter values from the population PK/PD model are shown in **Table 3.1**. Predicted (Bayesian posteriors) median AUCs at steady state ranged between 41.09 (10 mg/kg/day q24) and 487.5 mg*h/L (100 mg/kg/day q12).

Population PK/PD parameter *in vitro* model (free drug concentrations)			**Population PK/PD parameter *in vivo* model (total drug concentrations)**	
	Mean (SD)	Median	Mean (SD)	Median
Cl (L/h)	0.03 (0.02)	0.025	0.65 (0.2)	0.58
Vc (L)	0.5 (0.2)	0.44	0.86 (0.4)	0.56
Population PD parameter *in vitro* model			Population PD parameter *in vivo* model	
KgmaxS (log_{10} CFU/mL*h^{-1} or mg/L*h^{-1})	1.2 (0.3)	1.12	3.9 (3.6)	2.89
KgmaxR (log_{10} CFU/mL*h^{-1})	0.61 (0.5)	0.52	NA	NA
Popmax (CFU/mL or mg/L)	$2.5x10^9$($2.9x10^9$)	$1.5x10^9$	207.1 (18.7)	219.7
H	5.25 (2.9)	4.1	8.7 (7.2)	3.99
HR	7.03 (5.5)	3.8	NA	NA
KkmaxS (log_{10} CFU/mL*h^{-1} or mg/L*h^{-1})	1.3 (0.3)	1.25	0.21 (0.2)	0.14
Kimax	NA	NA	0.19 (0.006)	0.18
EC50S (mg/L)	7.99 (7.8)	5.96	6.93 (4.4)	6.07
EC50R (mg/L)	20.2 (15.6)	18.9	NA	NA
Kkim	NA	NA	0.02 (0.005)	0.02
IMAX	NA	NA	5.3 (0.6)	5.3
IC1 (CFU/mL)/(mg/L)	$4.8x10^4$ ($2.4x10^4$)	$4.1x10^4$	3.72 (3.9)	3.13
IC2 (CFU/mL)	163 (37)	98	NA	NA

Cl= Clearance; Vc=Volume of distribution in the central compartment; Kgmax= maximum rate of bacterial growth (in vitro model) ; Popmax= theoretical maximum bacterial density; H=Hill slope; C50= Vancomycin concentration producing half-maximal bacterial kill/CRP reduction; Kkim= maximum rate of CRP reduction induced by the immune system; IMAX=theoretical maximum rise of the immune system; Kimax=maximum rate of rise of immune system function; IC= initial condition in bacterial density/CRP (1 for the susceptible population and 2 for the resistant population). S and R correspond to the total susceptible population and the resistant bacterial population, respectively.

Table 3.1- Population PK/PD parameter values for both the vancomycin HFIM and the CLABSI rabbit model against CoNS infection.

Quantitative cultures from blood cultures were only intermittently positive for blood. The total bacterial density from the catheter tips at autopsy is shown in **Figure 3.6.**

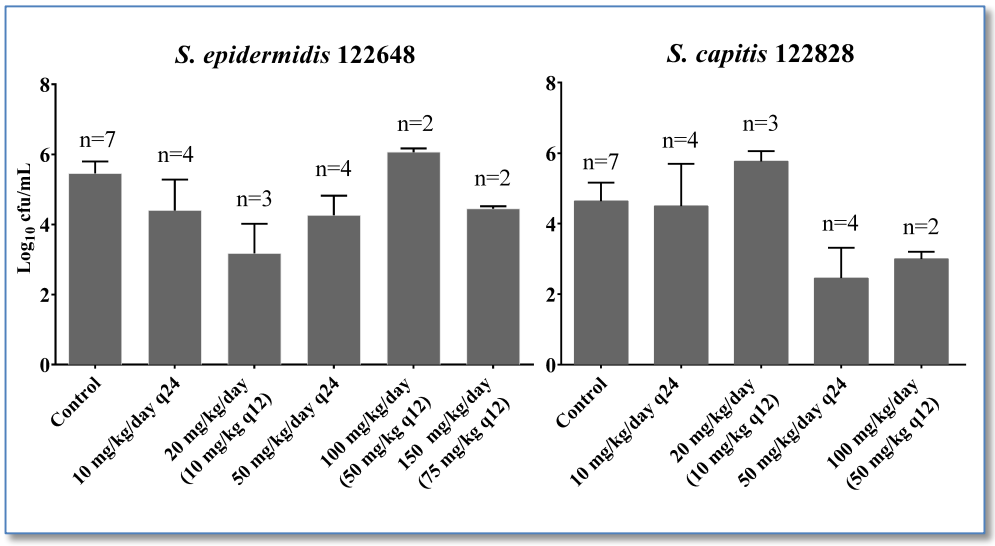


Figure 3.6- Central catheter tip cultures at autopsy from the CLABSI rabbit model.

(Pooled data means and standard error of means) for each of the dosage regimens and the strains investigated (*S. epidermidis* 122648 and *S. capitis* 122828) (Log_{10} CFU/mL).

The relationship between AUC:MIC and the CRP at the end of the experiment is shown in **Figure 3.7**. A total drug AUC:MIC at steady state of 76 mg*h/L was required to achieve a 50% decrease of CRP by 96 h (end of experiment). However, to achieve near maximal effect (80% decrease of CRP at 96 h), a total drug AUC:MIC of 520 mg*h/L was required for both isolates of CoNS **(Figure 3.7).**

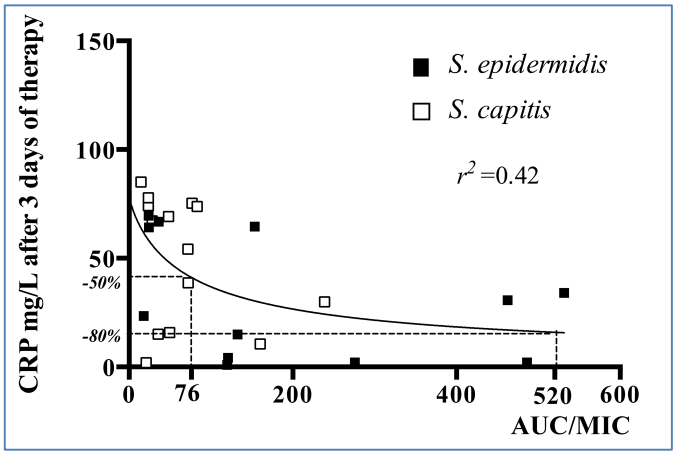


Figure 3.7- Pharmacodynamic target AUC/MIC ratio of vancomycin against CoNS in the rabbit CLABSI model.

3.4.6- Bridging Study

The distribution of expected CRP values in a population of 1000 simulated neonates for each PMA linked to their respective drug exposures (AUCs) receiving current dosage regimens showed that with standard therapy, neonates < 29 weeks PMA resulted to be under-dosed in comparison to the older age groups. The median predicted CRP from the simulations for neonates <29 weeks, 29-35 weeks, and > 35 weeks all receiving the currently recommended vancomycin regimen was 58.4, 49.53, and 49.23 mg/L, respectively. An optimized dosage regimen for the <29 weeks age group of 30 mg/kg/day (15 mg/kg q12h) reduced the CRP to values comparable to the ones of older age- groups (median 49.12 mg/L) **(Figure 3.8).**

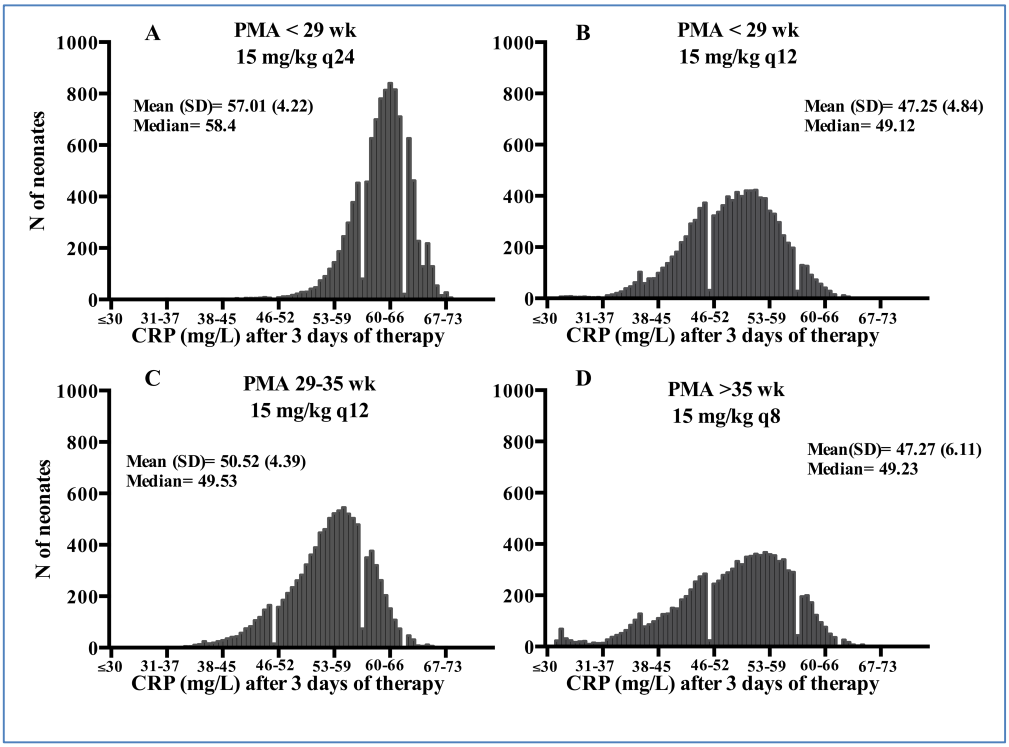


Figure 3.8- Bridging study in neonates. Predicted CRP-AUC linked histograms showing the distribution of simulated neonates per PMA age group.

(a) < 29 wk, (c) 29-35 wk and (d) > 35 wk, receiving currently recommended dosage regimens and a proposed optimized vancomycin regimen (b) 15mg/kg q12 for <29 week PMA-group. CRP means, standard deviations and medians are shown.

3.5- DISCUSSION

The inflammatory state that accompanies CoNS infection may contribute to poor neurodevelopmental outcomes in neonates (Adams-Chapman & Stoll 2006; Mitha et al. 2013). The aim of neonatal therapy is thus, to manage both infection and inflammation in the presence of an infected central line that ordinarily cannot be removed. The optimal vancomycin regimen to achieve this goal is not known, and is the focus of this study. We developed two new experimental models to investigate the PK/PD of vancomycin against CoNS, which yielded different, but complementary information.

The HFIM enables human neonatal concentration-time profiles to be simulated and is the ideal model to quantify bacterial killing and the emergence of antimicrobial resistance under drug pressure. However, its main weaknesses are the lack of an immune system and proteins for drug protein binding, which might have an impact on drug efficacy. In contrast, the rabbit CLABSI model is a closer mimic of neonatal infection and disease that employs CRP as the primary model readout to assess the response to antimicrobial therapy. While we acknowledge that several factors extraneous to drug exposure, such as immunological effectors and the inoculum may also have an impact on CRP concentrations we observed similar CRP concentration time profiles in rabbits and neonates receiving glycopeptide therapy (Figure 3.5). Furthermore, as is the case in human neonates in the NICU, blood cultures are only intermittently positive for CoNS in the rabbit CLABSI model, and infection causes inflammation without causing fulminant sepsis and death. All these attributes suggest the rabbit model is a faithful mimic of neonatal disease, and can be used for future assessment of antibiotics for neonates. This is despite the fact of the different vancomycin pharmacokinetics (mainly different drug clearance, see table

3.1) in the rabbits as opposed to the simulated neonatal clearance in the HFIM, which was corrected by dosing rabbits to aim clinically relevant drug exposures in terms of AUCs.

Studies conducted in the HFIM suggest that vancomycin exhibits concentration-dependent killing. Both the AUC:MIC and the C_{max}:MIC readily account for the experimental data when the total and resistant subpopulations were considered (Figure 3.4, Panels a and b). There is a progressive decline in bacterial density with increasing AUC:MIC. In contrast, a characteristic "inverted U" was observed when drug exposure was linked with the emergence of drug resistance (Tam et al. 2007; Zinner et al. 2003).The concentration-dependent effect of vancomycin on bacterial killing is consistent with other studies in MRSA and *S. pneumoniae* infections (Jenny Dahl Knudsen et al. 2000; Nicasio et al. 2012). The PK/PD data strongly suggest that the use of less fractionated regimens is appropriate in the clinic, with no pharmacodynamic evidence that vancomycin continuous infusions may be beneficial for treatment of CoNS. Furthermore, intermittent drug administration may facilitate the clinical use of vancomycin (by freeing up an i.v. line), minimize toxicity (because C_{min} is linked to toxicity)(Lodise et al. 2009) and minimize the emergence of drug resistance.

In the HFIM, the drug exposure that predicts near maximal killing and is required to suppress the emergence of drug resistance is a *f*AUC:MIC >400, which is equivalent to a *f*AUC > 800 mg*h/L (because the MIC of the study strains is 2 mg/L), and a total drug AUC of approximately 1330 mg*h/L (total AUC/MIC of 665) if 40% protein binding is assumed (Butterfield et al. 2011; De Cock et al. 2016). Such a value is higher than the total drug AUC:MIC value of 400 that is widely cited for treatment of invasive MRSA (Moise-Broder et al. 2004; Le et al. 2013; Frymoyer

et al. 2013; Liu, Bayer, Cosgrove, Daum, Fridkin, Gorwitz, Kaplan, Karchmer, Levine, Murray, Rybak, D. a. Talan, et al. 2011), and therefore raises the question of the clinical relevance of the experimental findings from the HFIM. The rabbit CLABSI model provides some additional context for this finding. Here, a total drug AUC:MIC of 520 (corresponding to a total drug AUC of 1040 (MIC = 2 mg/L) and a *f*AUC:MIC of 312) is required for near complete CRP suppression (Figure 3.7). The HFIM studies suggest this magnitude of drug exposure also prevents the emergence of CoNS mutants (Figure 3.2.B b and c).

Collectively therefore, both experimental models suggest: (1) higher AUC:MIC targets (total 665 and 520 for the HFIM and the rabbit model respectively) than are currently proposed for MRSA infection in adults are required for maximal bacterial kill, prevention of amplification of a resistant subpopulation and suppression of circulating CRP in the setting of a retained central line; and (2) the dose fractionation studies suggest that less fractionated regimens may be better in terms of bacterial kill and preventing the emergence of drug resistant subpopulations.

The recommended vancomycin regimens for neonates vary widely, but commonly used regimens in Europe are as follows: (1) 15 mg/kg/day q24h in < 29 weeks PMA (2) 15 mg/kg q12h in 29-35 weeks PMA and (3) 15 mg/kg q8h in >35 weeks PMA (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015b; Sharland et al. 2016). The bridging study provides an opportunity to examine the potential adequacy of these regimens. After examining the experimental data, we decided not to define a cut-off value to classify therapeutic success and failure because of the arbitrary nature of such a value. The absence of clinical data makes any split somewhat difficult to justify. Rather, we used the whole exposure-effect relationship obtained from the rabbit model, ultimately using a clinically relevant

readout of circulating CRP concentrations. Simulations suggest that neonates < 29 weeks PMA are under-dosed with respect to the older age- groups and have higher predicted CRP values. A regimen of 15 mg/kg q12h is needed to achieve a similar reduction in CRP concentrations compared with older neonates and infants.

This study provides the pharmacodynamic rationale to explore the use of higher dosages in neonates <29 weeks PMA. These ideas can now be tested in a multi-centre Phase IIb clinical trial. Somewhat surprisingly, the vancomycin exposure that is required for maximal anti-bacterial effect appears higher than is widely accepted for the treatment of more virulent Gram-positive organisms. The PK/PD data and models provide an insight into the difficulties of managing infection in critically ill neonates with a trade between bacterial killing, preventing emergence of drug resistance and minimizing drug related toxicity.

Chapter 4

Pharmacokinetics and Pharmacodynamics of Teicoplanin Against Methicillin Resistant *Staphylococcus aureus*

4.1- ABSTRACT

Objectives: There is limited information on teicoplanin pharmacodynamics to ensure the most optimal dose-exposure-effect relationships. The overall study aim was to identify the relevant pre-clinical PK/PD indices for teicoplanin to predict efficacy and suppression of resistance in MRSA infection.

Methods: A hollow-fibre infection model simulating a paediatric PK profile and a standardised neutropenic murine thigh infection model were developed. The PK/PD data generated was then co-modelled for each infection model using a non-parametric population modelling approach with Pmetrics. The posterior Bayesian estimates derived for each model were then used to study the exposure-effect relationships using a sigmoid Emax inhibitory model. Monte Carlo Simulations from previous developed population PK models in adults and children were used to explore the PTA for a variety of teicoplanin dosage regimens against current EUCAST wild-type susceptibility range.

Results: There was a concentration-dependent activity of teicoplanin in both the in *vitro* and *in vivo* models. A total *in vivo* AUC/MIC of 610.4 (total exposure of 305.2 mg*h/L) for an MRSA strain with an MIC of 0.5 mg/L was needed for efficacy (2 $\log_{10}$ cell kill) against a total bacterial population. A total AUC/MIC ratio of ~ 1500 (total exposure of ~ 750 mg*h/L) was needed to suppress the emergence of resistance.

Conclusions: This study improves our understanding on teicoplanin pharmacodynamics against MRSA and defines an *in vivo* AUC/MIC target for efficacy and suppression of resistance. Additional studies are needed to further corroborate the PK/PD index in a variety of infection models and in patients.

4.2- INTRODUCTION

Teicoplanin is a glycopeptide with a similar spectrum of antibacterial activity to vancomycin (The Electronic Medicines Compendium 2014; Cavalcanti et al. 2010). Teicoplanin is licensed in the EU and other countries for the treatment of moderate-to-severe methicillin-resistant Gram-positive bacterial infections in children and adults (The Electronic Medicines Compendium 2014). Teicoplanin was developed before contemporary PK/PD techniques and there is little pharmacodynamic information that provides a rationale for the optimal use of this agent.

Drug exposure targets that are associated with a high probability of a successful clinical outcome are relatively poorly defined. Initial regimens were designed to achieve a C_{min} of 5-10 mg/L (A. P. R. Wilson 2000). More recently, this drug exposure target has been increased to > 15 mg/L for the majority of clinical indications (e.g. bacteremia, pneumonia, complicated skin and soft-tissue infections). Higher targets of 20 and 30-40 mg/L are now advocated for the treatment of bone/joint infections, and infective endocarditis, respectively (The Electronic Medicines Compendium 2014). These updated targets are based on small retrospective studies in adults (Harding et al. 2000; Ueda et al. 2012). Herein, we describe the pharmacodynamics of teicoplanin against MRSA. We used a well-characterised hollow fibre infection model (HFIM) and a murine thigh infection model of MRSA to establish these relationships. We considered both the antibacterial effect of teicoplanin and the emergence of resistance to teicoplanin as primary study endpoints. We evaluated the relevant PK/PD indices that best described the killing of susceptible sub-populations and prevented the emergence of resistance. We finally bridged the experimental results to human patients to reflect on the adequacy of current EUCAST *in vitro* susceptibility breakpoints.

4.3- MATERIAL AND METHODS

4.3.1- Organism, Susceptibility Studies and Mutational Frequency

MRSA ATCC® 43300™(ATCC®, Middlesex, UK) was used for all experiments. The isolate was stored on beads at -80ºC. The MIC for the strain was determined using the EUCAST broth microdilution methodology on three separate occasions (European Committee on Antimicrobial Susceptibility Testing 2003). The mutational frequency of a subpopulation with MIC >=8 mg/L was determined using teicoplanin drug containing MH agar (Sigma-Aldrich, Dorset, UK), as previously reported (Nicasio et al. 2012).

4.3.2- Teicoplanin concentration determination

Concentrations of teicoplanin were measured using a commercially available fluorescence polarization immunoassay (FPIA; Thermo Fisher Scientific, Germany). This is a homogeneous particle-enhanced turbidimetric immunoassay that utilizes the Quantitative Microsphere System (QMS) technology and was implemented on an automated analyzer Abbott Architect ci4100. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody biding sites of the teicoplanin antibody reagent. A concentration-dependent agglutination inhibition curve was obtained with minimum and maximum rate of agglutination at the highest and lowest teicoplanin concentrations, respectively. The limit of quantification (LOQ) was < 3.0 mg/L. The dynamic range was 3-100 mg/L and overall (intra and inter-day) precision was < 6%. *This method was performed by Kevin Padmore*.

4.3.3- *In vitro* Model of MRSA Infection in a Hollow Fibre System

Teicoplanin for intravenous infusion (Targocid 400 mg, Sanofi Aventis, Surrey, UK) was used for all experiments. A paediatric pharmacokinetic profile was simulated in the HFIM. An elimination half-life of 6.4 hours for teicoplanin was targeted in all the experiments, which was based on a median clearance estimate from a previous population pharmacokinetic model in children (Ramos-Martin et al. 2014).

For each experiment, fresh bacterial isolates were grown on blood agar (Oxoid Ltd., Hants, UK) and incubated at 37º C for 24 hours. Bacteria were then inoculated into the extra-capillary space of each hollow-fibre (HF) cartridge. The desired starting inoculum (~6 $\log_{10}$ CFU/mL) was confirmed by quantitative culture on MH agar plates (Thermo Fisher Scientific, Runcorn, UK). The HFIM was incubated at 37ºC in ambient air.

4.3.4- *In Vitro* Pharmacokinetic and Pharmacodynamic Studies

Initial dose-finding studies were conducted using concentration-time profiles of teicoplanin corresponding to human dosages of 10-100 mg/kg/day. Dosages that encompassed the exposure-response relationships for bacterial killing and the emergence of resistance were defined in preliminary experiments.

A total daily dose of 10 and 30 mg/kg/ day of teicoplanin was administered in the following ways: (a) a bolus administered every 24 hours (q24h); (b) two half dosages administered q12h; and (c) the total daily dose infused over a 24 h period (CI). Treatment was initiated 24 hours post-inoculation. Experiments were conducted for up to 7 days to simulate the typical duration of clinical therapy and provide the opportunity to observe the emergence of antimicrobial resistance.

4.3.5- *In vitro* Pharmacokinetic Studies

PK samples (1 mL) were withdrawn from the central compartment of the HFIM before each dose and at 1, 3, 6, 12 and 24 hours. Sampling occurred during the first dose interval and at day 6 of therapy. The area under the teicoplanin concentration time curve (AUC_{0-24}), maximum concentration (C_{max} or peak) and minimum (C_{min}) teicoplanin concentrations at steady state (144-168 h), were calculated from the Bayesian posterior estimates for the PK parameter values from each fibre (see below).

4.3.6- *In vitro* Pharmacodynamic studies

Bacterial samples (1 mL) were withdrawn from the extra-capillary space of each HF cartridges and ten-fold serial dilutions (100μL) were plated to both free and drug-containing agar plates. Sampling occurred at 0, 2, 6 and 24 hours post-infection and every 24 hours thereafter (immediately prior to dosing). Total and resistant subpopulations were quantified. To investigate whether the mutants that grew on drug-containing plates had an elevated MIC, approximately 10 colonies were selected from each plate and the MIC was re-estimated as previously described.

4.3.7- Pharmacokinetic and Pharmacodynamic Studies in a Murine Thigh Infection model

A well-characterised neutropenic murine thigh infection model was used to provide a complementary perspective on the pharmacodynamics of teicoplanin to that provided by the HFIM. All experiments were conducted under UK Home office project

license PPL 40/3630 and approved by the University of Liverpool's Animal Welfare and Ethics Review Board.

Male CD1 mice (16-20 g) were rendered neutropenic on day -4 and -1 with intra-peritoneal administration of cyclophosphamide (Baxter, Liverpool, UK) (150 and 100 mg/kg, respectively)(Zuluaga, Salazar, C. A. Rodriguez, et al. 2006). On day 1, mice were inoculated with 2 x 10^6 CFU/mL MRSA (43300) in each posterior thigh muscle in a 50 μL volume. Teicoplanin was commenced 2 h post-infection and was administered i.v. every 12 hours.

4.3.8- *In vivo* Pharmacokinetic studies

Dosages of 2.5, 15 and 100 mg/kg/day were chosen to investigate the total bactericidal effect and the suppression of resistance based on preliminary dose-finding studies. PK sampling was performed at: 0, 0.5, 1, 2, 4 and 12 hours post-dose. Three mice were used per dose-time-point. Samples were immediately spun and plasma stored at -80ºC until analysis.

4.3.9- *In vivo* Pharmacodynamic studies

At the time of sacrifice (at 2, 12 and 26 h post-infection) both thighs were aseptically removed and placed in separate culture tubes with 2 mL PBS. Samples were individually homogenised using a polytron disperser VDI 12 (VWR, Lutterworth, UK). One hundred μL aliquots from 10-fold serial dilutions were plated to drug free and MH agar containing teicoplanin 8 mg/L. The mean value of the bacterial burden from the

left and right thigh from each mouse was determined. The mean and SD of a group of three mice was then calculated.

4.3.10- PK/PD Mathematical Modelling

A mathematical model was fitted to the experimental data from the HFIM and mice. The population PK program Pmetrics was used for all fitting (v.1.2.9, University of Southern California, Los Angeles, CA)(Neely et al. 2012) for R (version 3.1.0, Institute for Statistics and Mathematics, Vienna, Austria)(R Team 2013). The structural model took the form:

(1) $\frac{dX1}{dt} = R(1) - \left(\frac{CL}{Vc}\right) * X1$

(2) $\frac{dx2}{dt} = KgmaxS * X2 * \left(1 - \frac{(X2+X3)}{POPmax}\right) - \left(\frac{KkmaxS * x2 * \left(\frac{x1}{Vc}\right)^{Hs}}{(C50S^{Hs} + \left(\frac{x1}{Vc}\right)^{Hs}}\right)$

(3) $\frac{dx3}{dt} = KgmaxR * X3 * \left(1 - \frac{(X2+X3)}{POPmax}\right) - \left(\frac{KkmaxR * x3 * \left(\frac{x1}{Vc}\right)^{HR}}{(C50R^{HR} + \left(\frac{x1}{Vc}\right)^{HR}}\right)$

Equation 1 describes the rate of change of the amount of teicoplanin (mg) in the central compartment (X1). **Equation 2 and 3** describe the rate of change of burden of a susceptible bacteria population (S) and a resistant/mutant (R) bacteria population in the HFIM/murine model. The rate of growth is a balance between bacterial growth and death. POPmax (CFU/mL) is the theoretical maximum bacterial density; KgmaxS/R (Log_{10}CFU/mL/h) is the maximum rate of bacterial growth in both subpopulations; $Kkmax_{S/R}$ (Log_{10}CFU/mL/h) is the rate of bacterial killing induced by teicoplanin; C50S/R (mg/L) represent the teicoplanin drug concentrations that produce half-maximal killing in both sub-populations; HS/HR are the respective slope functions.

There were three output equations for the model: (1) Y(1)=X(1)/V, which described the time course of teicoplanin concentrations; (2) Y(2)=DLOG10(X(2)+X(3)), which described the time course of the total population; and Y(3)=DLOG10(X(3)), which described the time course of the resistant subpopulation.

The goodness of fit of each model to the data was assessed using a combination of the following: (1) the log-likelihood value, (2) the Akaike information criterion (AIC), (3) the coefficient of determination (r^2) from the linear regression of the observed-predicted plots before and after the Bayesian step, and (4) a minimization of bias and imprecision values of the observed-predicted plots.

4.3.11- Exposure-effect relationships and optimal targets

To determine the PK/PD index that best described bacterial killing and the suppression of drug resistance, scatter plots from the *in vitro* dose-fractionation studies, were constructed that related the AUC:MIC, C_{max}:MIC and C_{min}:MIC with both the observed antibacterial effect and the emergence of a drug resistant subpopulation from the HFIM. The relevant PK/PD index was then further explored with the *in vivo* mice data. A non-linear regression model was fitted to the data using Prism software (GraphPad Software Inc., La Jolla, CA). The coefficient of determination and visual examination of the fit was used to discriminate the various pharmacodynamic indices.

4.3.12- Monte Carlo Simulations and Probability of Target Attainment (PTA) analysis

Monte Carlo simulations were performed with Pmetrics. A population of 1,000 simulated patients was generated for each teicoplanin regimen. These simulated patients were used to examine the outcomes of candidate regimens of teicoplanin. For the adult simulations we used the population PK estimates and covariance matrix from a previously developed population PK model (Ramos-Martin et al. 2014). For the paediatric simulations, we used the population PK estimates and covariance matrix from a previously developed PK model in children (submitted for publication). For children, the weight-based dose of teicoplanin (mg per kg) was administered to each simulated patient as a 3-minute i.v. infusion. The weight-based dosage was converted to an absolute dosage for i.v. administration (as would happen at the bedside) by multiplying by the simulated weight (for example, a 20 kg child receiving 10 mg/kg would receive 200 mg of teicoplanin). The following covariate limits were used for the simulations: weight: 9-62.2 kg; age: 1-16 years old and estimated GFR (Schwartz): 49-178.1 mL/min/1.73 m^2). A variety of candidate regimens were investigated: the standard teicoplanin dosing and regimens 2 and 3 with the same daily maintenance dose but different frequency of dosing (q24h vs q12h). We considered this relevant to evaluate the impact of the frequency in AUC and C_{min} attained drug exposures. Estimation of the drug exposures in terms of AUC_{0-24} and C_{min} at steady state (between days 3 and 4 of treatment) were performed for each dosage regimen for both adults and children.

For the PTA analysis, a target AUC/MIC (stasis and 2 $\log_{10}$ cell kill in the murine model) was used. The distribution of the MRSA MICs determined using

EUCAST methodology from 0.032 to 16 mg/L were plotted (European Committee on Antimicrobial Susceptibility Testing 2010). Fractional target attainment was calculated for the various regimens to identify the achievement of the AUC/MIC targets by comparing the PTA against the teicoplanin MIC distribution for MRSA.

4.4- RESULTS

4.4.1- *In vitro* susceptibility and mutational frequency

The modal teicoplanin MIC for the MRSA ATCC 43300 was 0.5 mg/L. The strain was oxacillin resistant by E-test (Oxoid). The mutational frequency (at 16 x MIC) was 1.02 x 10^{-7}. MICs from resistant mutants at the end of *in vitro* experiments (168 h) were up to five two-fold dilutions higher (2 to ≥ 16 mg/L).

4.4.2 -Pharmacodynamics of Teicoplanin against MRSA: Dose Finding Studies and Dose Fractionation Studies in the HFIM

There was a dose-dependent decline in the total bacterial density following exposure to teicoplanin. Total bactericidal effect was achieved with dosages of ≥ 10 mg/kg/day q24h and suppression of resistance with dosages ≥ 30 mg/kg/day q24h (**Figure 4.1.A**). The estimated $t_{1/2}$ of teicoplanin in the HFIM was 8.7 hours.

In **figure 4.1.B** the population PK/PD final model observed versus predicted plots are shown. The r^2 of the linear regression were 0.9 and 0.7 for the PK and PD, respectively, after the Bayesian step.

The results from the dose fractionation studies are shown in **Figure 4.2**. Non-linear regression analysis showed a strong correlation between fC$_{max}$: MIC, fAUC:MIC and fC$_{min}$:MIC and bacterial killing (r^2 = 0.92, 0.9 and 0.86 respectively). There was also a strong association between fC$_{max}$:MIC and fAUC:MIC and suppression of teicoplanin resistance (r^2 =0.79, 0.95 respectively). In contrast, a weaker relationship was apparent between fC$_{min}$:MIC and suppression of teicoplanin resistance (r^2 =0.45).

In the HFIM, a teicoplanin fAUC/MIC ratio at steady state ≥ 576 mg*h/L was associated with a 2 $\log_{10}$ CFU/mL decline in bacterial density and ≥ 1325 was required

to suppress the emergence of resistant clones (**Figure 4.2.a**). For the resistance studies, a characteristic "inverted U" was observed with maximal amplification of resistance with a *f*AUC:MIC of approximately 400-600 mg*h/L (**Figure 4.2.a**).

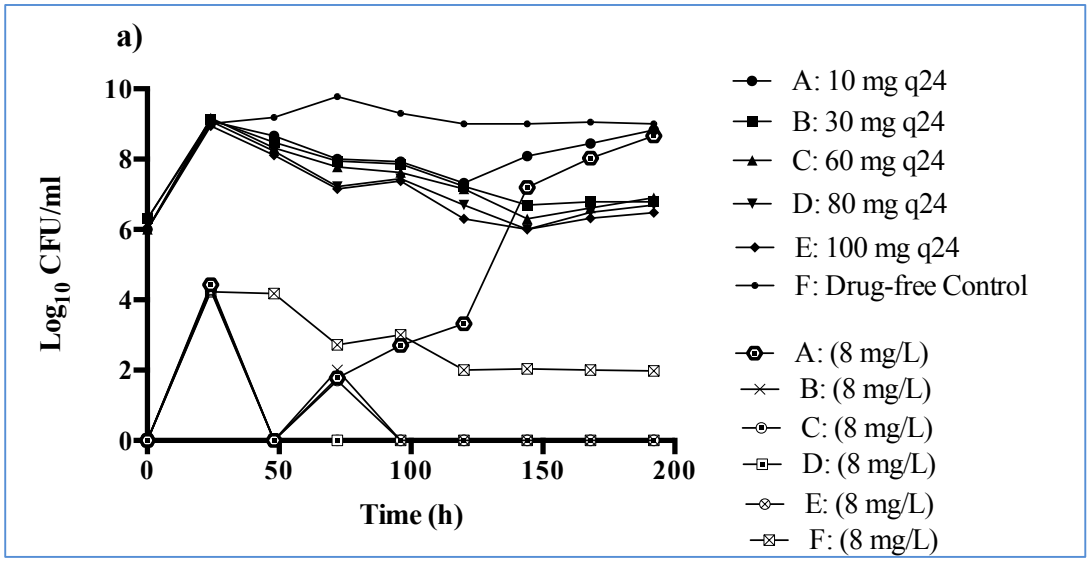


Figure 4.1.A- Dose finding studies results in the HFIM showing the total bactericidal effect of teicoplanin against MRSA with dosages ≥30 mg/kg/day and the emergence of drug resistance with 10 mg/kg/day.

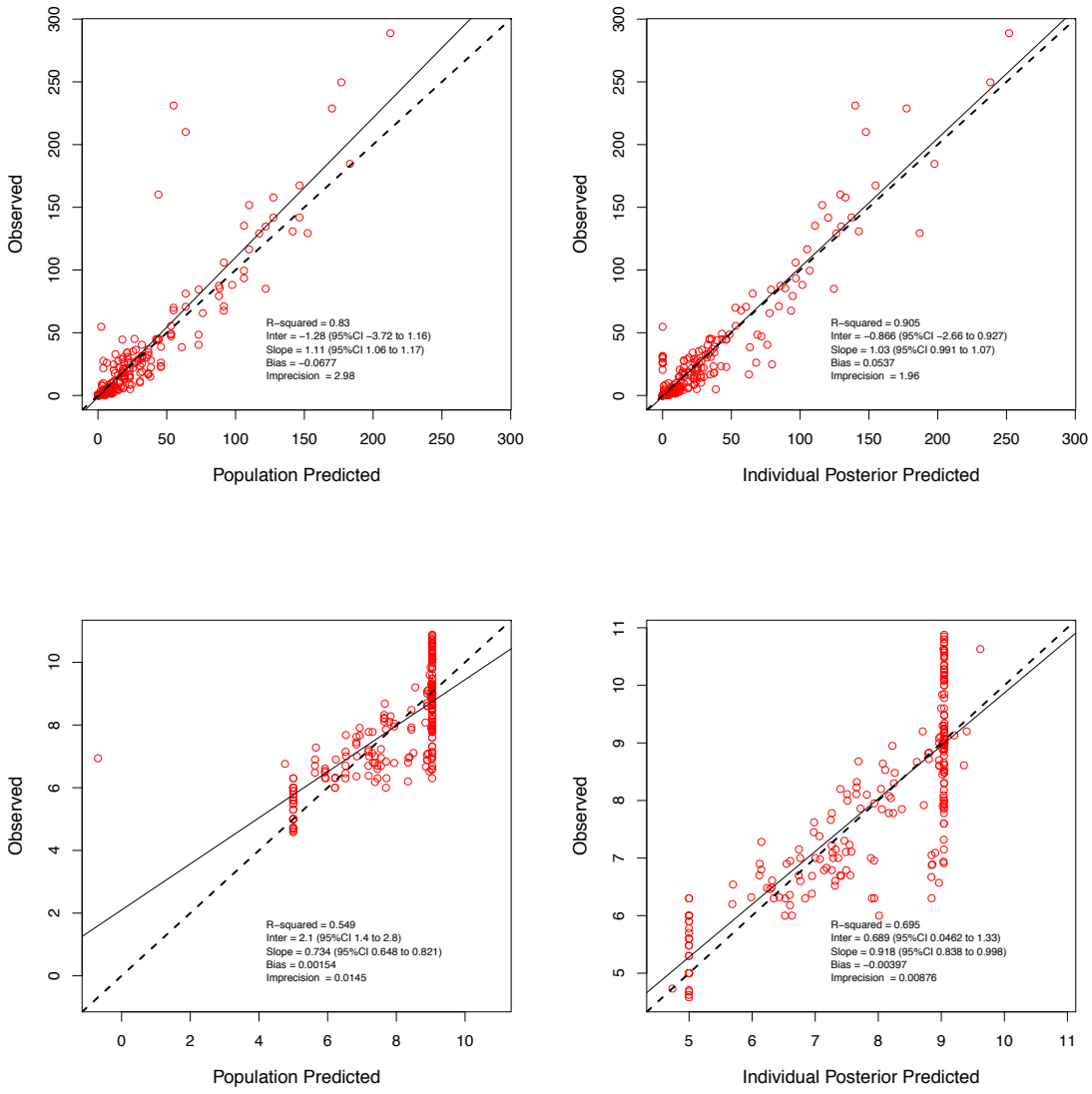


Figure 4.1.B- Population teicoplanin PK/PD model from the HFIM showing the observed *versus* predicted PK concentrations (mg/L) (top) and PD total bacterial densities (log_{10} CFU/mL) (bottom) plots, respectively, for the population and the individual posterior predictions.

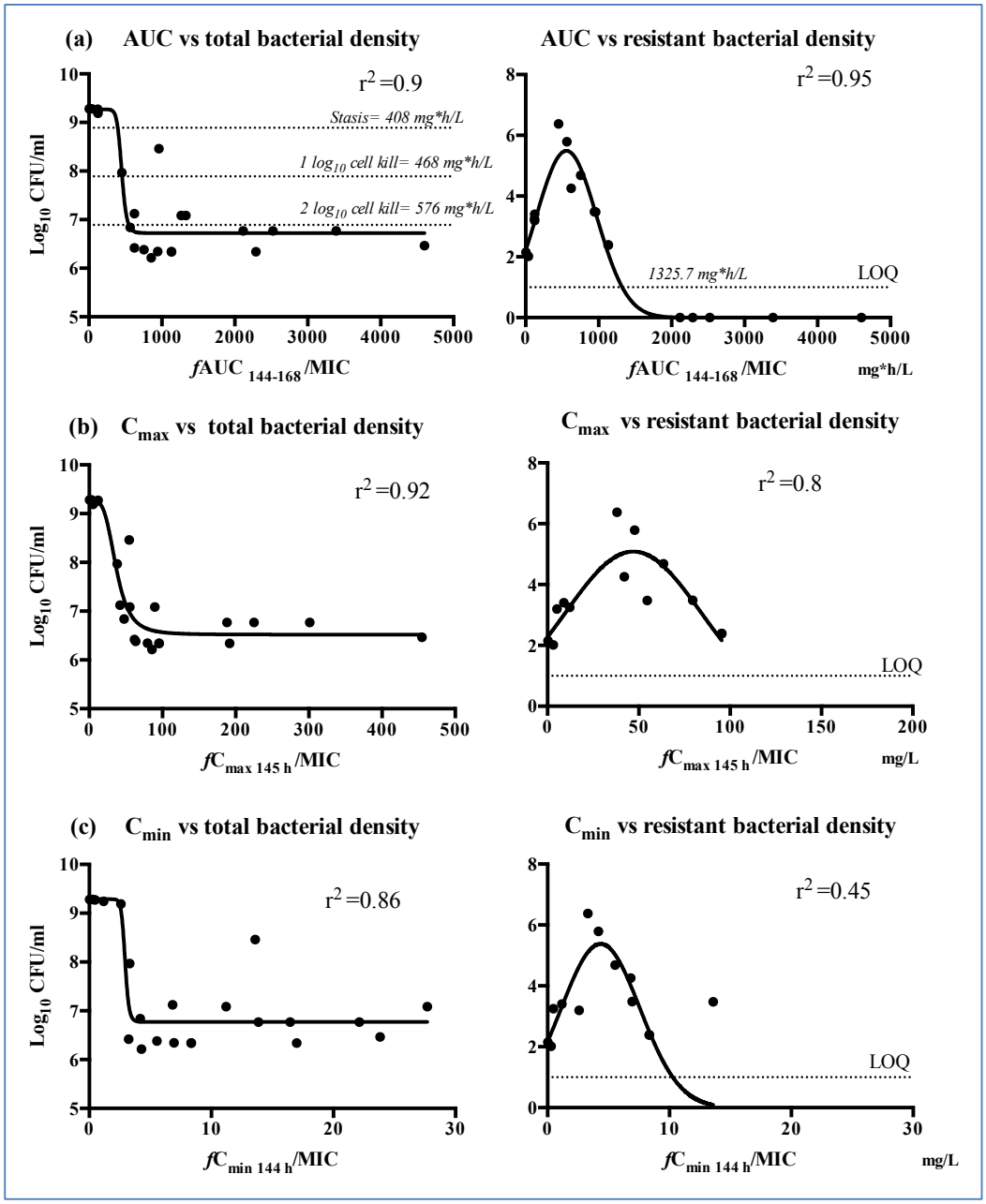


Figure 4.2- Teicoplanin pharmacodynamics against MRSA in the HFIM.

An Emax sigmoid model was fitted to the data (Bayesian individual posterior estimates from the population PK-PD model). a) *f*AUC (144-168 h) *vs* total and resistant bacterial density at the end of therapy (168 h); b) fC_{max} (145 h) *vs* total and resistant bacterial density at the end of therapy and c) fC_{min} (144 h) *vs* total and resistant bacterial density at the end of therapy. On the right column, the effect against the resistant bacterial populations is shown. A dashed line shows the limit of quantification (LOQ) for bacterial load.

4.4.3- Murine MRSA Neutropaenic Thigh Infection Model

There was a dose-dependent decline in total bacterial density in the thighs of mice treated with teicoplanin. Teicoplanin was bactericidal (≥ 2 $\log_{10}$ cell kill) for all regimens. There was emergence of a resistant sub-population with all regimens investigated with the appearance of an inverted U (**Figure 4.3b**).

Based on the HFIM studies (above), the AUC:MIC was used as the dynamically linked index for both effect and the emergence of resistance in the mouse (**Figure 4.3**). A teicoplanin total drug AUC_{0-24}/MIC ratio of ≈ 88.8 and 610.4 mg*h/L was associated with stasis and near maximal bacterial killing (2 $\log_{10}$ cell kill), respectively. A total drug AUC_{0-24}:MIC ratio ≥ 1400-1500 mg*h/L was required for near complete suppression of emergence of resistance. Maximal amplification of resistance was seen with total drug AUC_{0-24}/MIC ratios of 500-700 mg*h/L (**Figure 4.3b**).

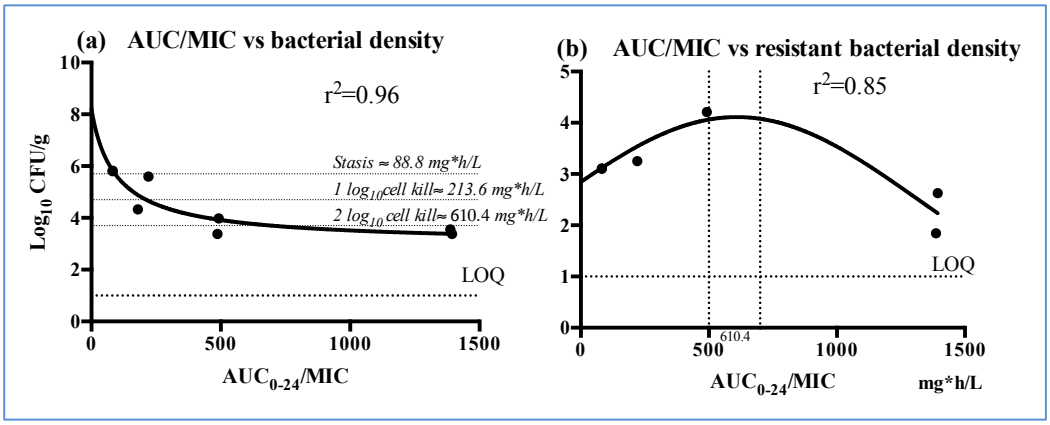


Figure 4.3- Teicoplanin total AUC/MIC ratio against MRSA total and resistant bacterial density from the 26 h mouse thigh infection model.

Each symbol (filled circle) represents the mean of data for three mice. Effect=8.23-((5.24x (AUC:MIC)$^{0.94}$)/((AUC:MIC)$^{0.94}$+98.5 $^{0.94}$). Figure 4.2(a) shows the concentration-dependent decline in the total bacterial density reaching ≥ 2 $\log_{10}$ cell kill CFU/g after AUC/MIC ratios of 600 mg*h/L. Thin dashed lines represent the mean bacterial load at the start of therapy (stasis) and 1 and 2 $\log_{10}$ drop cell kill. Thick dash line represents the LOQ. Figure 4.2(b) shows the characteristic "Inverted U" phenomenon in the resistant subpopulation with amplification of resistance at AUC/MIC ratios between ~500-700 mg*h/L and near maximal effect at ≥1500 mg*h/L.

The population PK/PD observed versus predicted plots from the mouse neutropaenic model are shown in **figure 4.4**. The r^2 of the linear regression after the Bayesian step were 0.99 and 0.95 for the PK and PD, respectively. The population PK/PD model parameter estimates from both pre-clinical models are summarised in **table 4.1.**

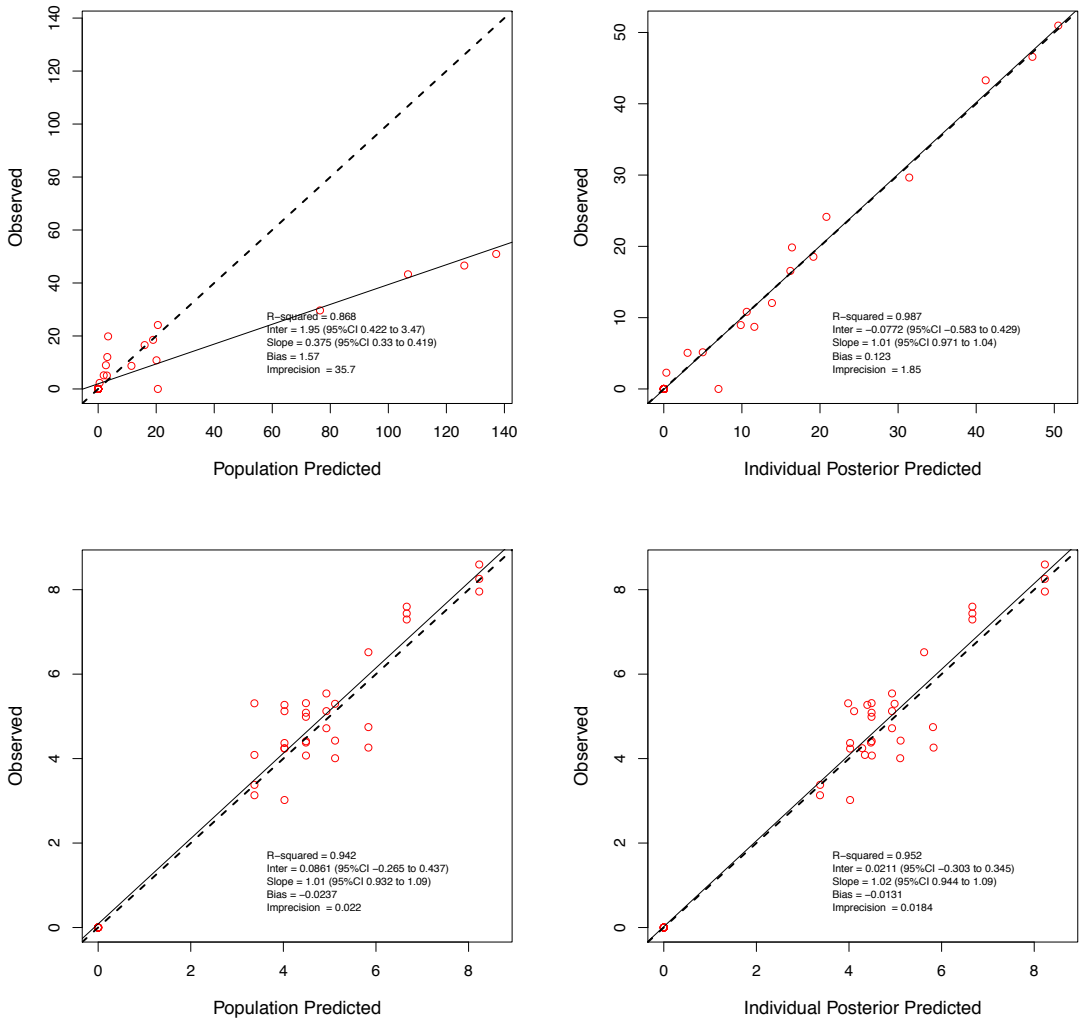


Figure 4.4- Population PK/PD model observed *versus* predicted plots for the *in vivo* mouse neutropaenic thigh infection model showing the predicted population and individual teicoplanin PK concentrations (mg/L)(top) and PD total bacterial densities (log $_{10}$ CFU/mL)(bottom).

4.4.4 Monte Carlo Simulations and PTA analysis

The PTA analyses showed that adult and paediatric patients receiving a standard dosage regimen for a target of a total AUC/MIC ratio of 610.4 were only successfully treated (≥ 90% probability of success) if the MIC of the strain was ≤ 0.125 mg/L in adults and ≤ 0.064 in children. The achievement of AUC/MIC targets required for *in vivo* stasis (AUC/MIC 88.8) allowed a successful treatment of strains with MICs ≤ 1 in adults and ≤ 0.5 in children. If an increased teicoplanin dosage was used along with a pharmacodynamic target of 88.8, then a satisfactorily high probability of target attainment was achieved for MIC values ≤ 2mg/L in both adults and children. The PTA plots and fractional target attainment are shown in **figure 4.3**.

Calculations of drug exposures simulated at steady state (days 3-4) in terms of $AUC_{0\text{-}24}$ and C_{min} are shown in **table 4.2** for each population (adults and children) and dosage regimen simulated.

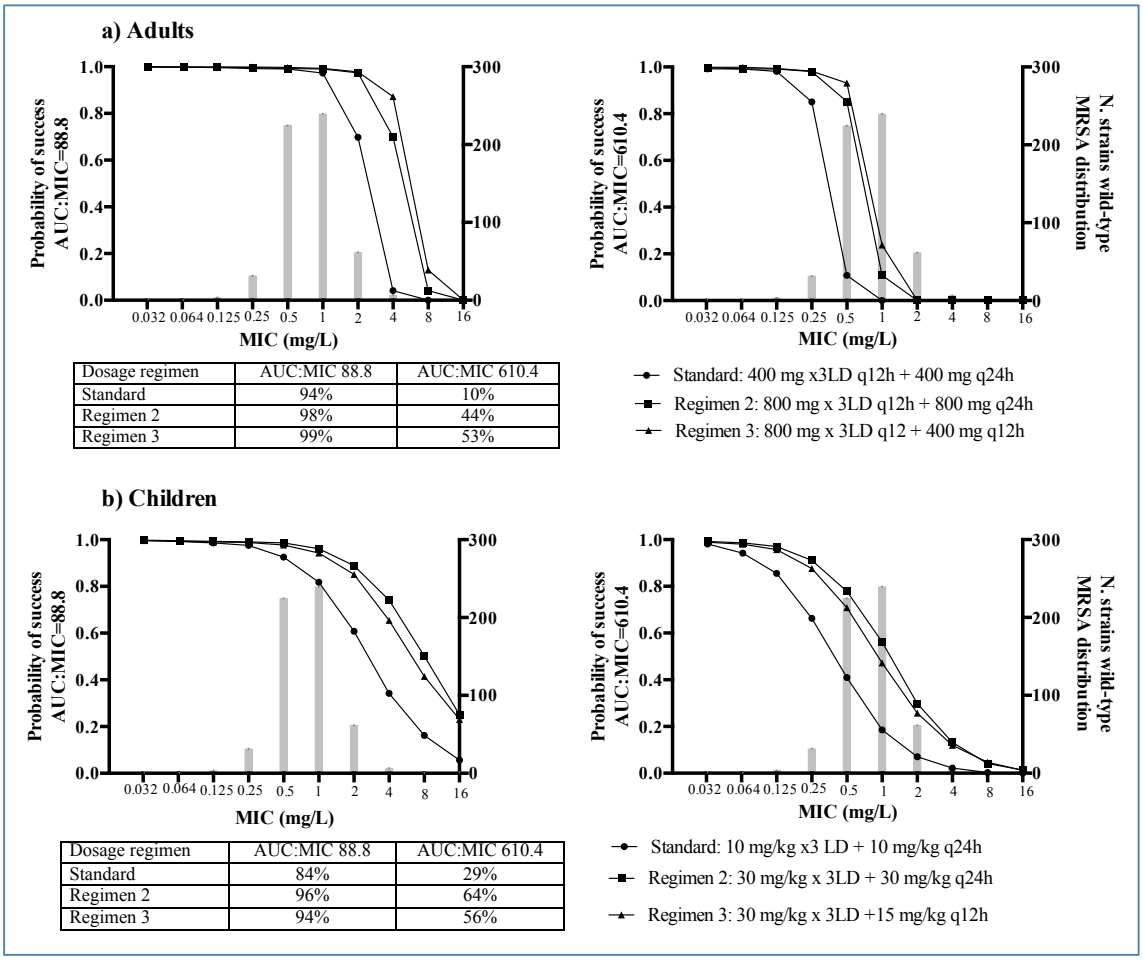
Population PK/PD parameter *in vitro* model (free drug concentrations)			**Population PK/PD parameter *in vivo* model (total drug concentrations)**	
	Mean (SD)	Median	Mean (SD)	Median
Cl (L/h)	0.04 (0.02)	0.04	0.08 (0.05)	0.06
Vc (L)	0.5 (0.2)	0.5	0.6 (0.4)	0.34
Population PD parameter *in vitro* model			Population PD parameter *in vivo* model	
KgmaxS (log_{10} CFU/mL *h^{-1})	0.62 (0.19)	0.68	0.42 (0.03)	0.4
KgmaxR (log_{10} CFU/mL*h^{-1})	0.15 (0.09)	0.2	0.09 (0.06)	0.06
Popmax (CFU/mL)	1.6^10^9 (1.2^10^9)	1.3^10^9	1.3^10^8(8^10^7)	2^10^8
H	8.6 (2.6)	9.9	13.7 (3.7)	15.1
HR	13.9 (2.6)	16	14.1 (5.2)	17.1
KkmaxS (log_{10} CFU/mL*h^{-1})	0.7 (0.2)	0.7	0.52 (0.06)	0.51
KkmaxR (log_{10} CFU/mL*h^{-1})	0.7 (0.4)	0.4	0.75 (0.15)	0.79
EC50S (mg/L)	11.6 (3.6)	11.8	1.2 (0.3)	1.2
EC50R (mg/L)	40.2 (9.9)	38.03	64.1 (9.8)	70
IC1 (CFU/mL)	9.8^10^4 (9.4^10^4)	10^10^3	4^10^4 (8^10^3)	3.6^10^4
IC2 (CFU/mL)	20.2 (15.2)	13.04	159 (162)	206

Cl= Clearance; Vc=Volume of distribution in the central compartment; Kgmax= maximum rate of bacterial growth; Popmax= theoretical maximum bacterial density; H=Hill slope; C50= Teicoplanin concentration producing half-maximal bacterial kill; IC= initial condition in bacterial density (1 for the susceptible population and 2 for the resistant population). S and R correspond to the total susceptible population and the resistant bacterial population, respectively. In the murine model: CFU/mL corresponds to CFU/g.

Table 4.1- Population PK and PD parameter estimates from the HFIM and the neutropaenic mice thigh model.

Dosage regimen simulated	AUC_{72-96} median (IQR) (mg*h/L)	C_{min} 96 h median (IQR) (mg/L)
Adults		
1) Standard: 400 mg x 3LD q12h +400 mg q24h	205 (170.9-254.4)	8.72 (7.23-10.8)
2) 800 mg x 3LD q12h + 800 mg q24h	410.7 (342.1-509.6)	17.4 (14.5-21.6)
3) 800 mg x 3LD q12h + 400 mg q12h	499.03 (413.4-600.4)	20.96 (17.2-25.5)
Children		
1) Standard: 10 mg/kg x 3LD q12 + 10 mg/kg q24h	237.4 (114-475.5)	5.4 (1.7-13.4)
2) 30 mg/kg x 3LD q12h+30 mg/kg q24h	711.4 (342.1-1426.2)	16.2 (5.1-40.1)
3) 30 mg/kg x 3LD q12h+15 mg/kg q12h	560.05 (258.7-1298.6)	19.7 (7.9-47.7)

Table 4.2- Simulated drug exposures (AUC_{72-96} and $C_{min\ 96}$) in adults and children between days 3 and 4 of therapy for standard and dosage increased regimens.



Dosage regimen	AUC:MIC 88.8	AUC:MIC 610.4
Standard	94%	10%
Regimen 2	98%	44%
Regimen 3	99%	53%

Dosage regimen	AUC:MIC 88.8	AUC:MIC 610.4
Standard	84%	29%
Regimen 2	96%	64%
Regimen 3	94%	56%

Figure 4.5- Monte Carlo Simulations and PTA analysis.

a) PTA in adult patients receiving standard and increased dosage regimens for a target AUC/MIC of 610.4 and 88.8 mg*h/L by day 3-4 of therapy; b) PTA in paediatric patients (1-16 years old) with standard and increased dosage regimens for a target AUC/MIC of 610.4 and 88.8 mg*h/L. The underlying grey bars on each plot represents the wild-type EUCAST reported distribution for teicoplanin against MRSA. The tables show the fractional target attainment for each of the simulated dosage regimens against the MRSA teicoplanin MIC distribution.

4.5- DISCUSSION

In this study the AUC:MIC ratio was the pharmacodynamic index that best links the administration of teicoplanin with the antibacterial effect and the emergence of resistance. A total drug AUC/MIC ratio of 88.8 and 610.4 mg*h/L results in stasis and a 2-log cell kill, respectively. A previous study in mice also suggests teicoplanin displays concentration-dependent antibacterial activity with both C_{max}/MIC and %T>MIC identified as the relevant pharmacodynamic indices (J D Knudsen et al. 2000). We demonstrate that all pharmacodynamic targets result in the amplification of resistant mutants (Figure 4.2). Thus, the antibacterial effect resulting from clinically relevant drug exposures is always tempered by the emergence of drug resistance, and the two are inextricably related. Higher teicoplanin exposure thresholds (e.g. AUC:MIC ≈ 1500) that are predicted to prevent the emergence of resistance are not obtainable using current regimens.

The magnitude of drug exposure that produces stasis in immunocompromised mice is relevant for patients with *S. aureus* bacteraemia (oritavancin and linezolid), and complicated skin and skin structure infections (linezolid and tigecycline)(Bhavnani et al. 2006; Boylan et al. 2003; Rayner et al. 2003; Andes et al. 2002; Ambrose et al. 2007; van Ogtrop et al. 2000). A teicoplanin AUC:MIC of 88.8 is likely relevant for patients with skin and skin structure infections. As it can be seen from Figure 4.3, such a target is readily achievable for simulated patients receiving currently licensed regimens with fractional target attainment rates of 84% and 94% in children and adults, respectively. For more serious staphylococcal infections, such as pneumonia, a higher pharmacodynamic target is generally required (Ambrose et al. 2003; Ambrose et al. 2007). An AUC:MIC of 610.4

produces logarithmic bacterial killing in mice. This estimate is consistent with several retrospective clinical studies in which teicoplanin AUCs ≥ 750-800 mg*h/L and AUC:MIC≥ 900 are associated with favourable clinical outcomes in adults with serious MRSA infections (Hagihara et al. 2012; Takeda et al. 2016b; Kanazawa et al. 2011). A higher AUC:MIC ratio is also consistent with the recent upward revision in target C_{min} concentrations recommended in the SPC for the treatment of patients with deep-seated staphylococcal infections (The Electronic Medicines Compendium 2014). The difficulty of achieving these higher exposure targets with currently recommended regimens is highlighted by the fact that fractional attainment rates are only 10% and 29% of simulated adults and children, respectively.

The administration of a fixed teicoplanin regimen is reasonable for skin and skin structure infections. Standard teicoplanin therapy results in target attainment in a majority of patients and the wild-type population can be largely covered. In contrast, more serious infections that require higher drug exposures require routine TDM. Too few patients receiving a fixed regimen achieve an adequate AUC:MIC of 610.4 and are at risk of concentration-dependent therapeutic failure. For these patients, drug measurement and dosage adjustment is required. An alternative strategy is the use of combination chemotherapy. There is a large amount of preclinical data that has explored the potential benefits of combining glycopeptides with rifampicin, fosfomycin and β-lactams (de Matos et al. 2014; Tang et al. 2012; Leonard 2012; Werth et al. 2013; Dilworth et al. 2014). These ideas are being studied in several prospective clinical trials. ARREST (ISRCTN 37666216) is evaluating the potential benefit of adjunctive rifampicin in *S. aureus* bacteraemia (Thwaites et al. 2012) and CAMERA-2 (NCT02365493) is investigating the combination of glycopeptides with β-lactams to treat hospitalised patients with MRSA bacteraemia (Tong et al. 2016).

Monte Carlo simulations and the PTA analysis suggest that patients infected with strains at the upper end of the wild type distribution may be difficult to treat. This appears to be the case even if a stasis endpoint is used. The ECOFF using EUCAST methodology is 2 mg/L (European Committee on Antimicrobial Susceptibility Testing 2010). At this MIC, only 60% and 70% of children and adults, respectively, receiving a standard regimen is predicted to achieve a stasis target. This is strong evidence to consider a reduction in the breakpoint by at least a dilution to 1 mg/L (Turnidge & Paterson 2007). For more serious infections that require higher pharmacodynamic targets the situation is worse and the breakpoint divides the wild-type population.

We must acknowledge that only a single strain was studied in the murine model and HFIM. Nevertheless, two experimental models were used that yielded complementary information and allowed us to exploit the respective strengths of each. The murine thigh model is well characterised, has the advantage of simulating protein binding and is a mimic of complicated skin and skin structure infections that are common clinical problems. In contrast, the hollow fibre infection model enabled us to explore the pharmacodynamic relationships important for the emergence of resistance. Together, the findings from these two model systems and pharmacodynamic analyses suggest that teicoplanin should be used with some caution for the treatment of strains with MICs at the upper edge of the wild type population. Therapeutic drug monitoring is a requirement for the treatment of serious infections to minimise the probability of concentration dependent therapeutic failure.

Chapter 5

Population Pharmacokinetics of Teicoplanin in Children

5.1- ABSTRACT

Objectives: Teicoplanin is frequently administered in children to treat Gram-positive bacterial infections. However, little is known about the pharmacokinetics (PK) of teicoplanin in children to justify the optimal dosing regimen. The aim of this study was to determine the population PK of teicoplanin in children and evaluate current dosage regimens.

Methods: A PK hospital-based study was conducted. Current dosage recommendations were used for children up to 16 years of age. Thirty-nine children were recruited. Serum samples were collected at first dose interval (1, 3, 6 and 24 h) and at steady state. A standard 2-compartment PK model was developed, followed by structural models that incorporated weight. Weight was allowed to affect clearance (CL) using linear and allometric scaling terms.

Results: The linear model best accounted for the observed data and was subsequently chosen for Monte Carlo simulations. The PK parameters medians/means (SD) were: CL= [0.019/0.023 (0.01)]*weight (L/h/kg), Volume 2.282/4.138 (4.14) (L), Kcp 0.474/3.876 (8.16) (h^{-1}), Kpc 0.292/3.994 (8.93) (h^{-1}). The percentage of patients with C_{min}< 10 mg/L was 53.85%. The median/mean (SD) total population area under the concentration-time curve (AUC) was 619/527.05 (166.03) mg*h/L. Based on Monte Carlo simulations, only 30.04 % (median AUC = 507.04), 44.88 % (494.1) and 60.54% (452.03) of patients weighting 50, 25 and 10 kg respectively, attained trough concentrations >10 mg/L by day 4 of treatment.

Conclusions: Teicoplanin population PK is highly variable in children with a wider spread of AUC distribution as compared with adults. Therapeutic drug monitoring should be a routine requirement to minimize sub-optimal concentrations.

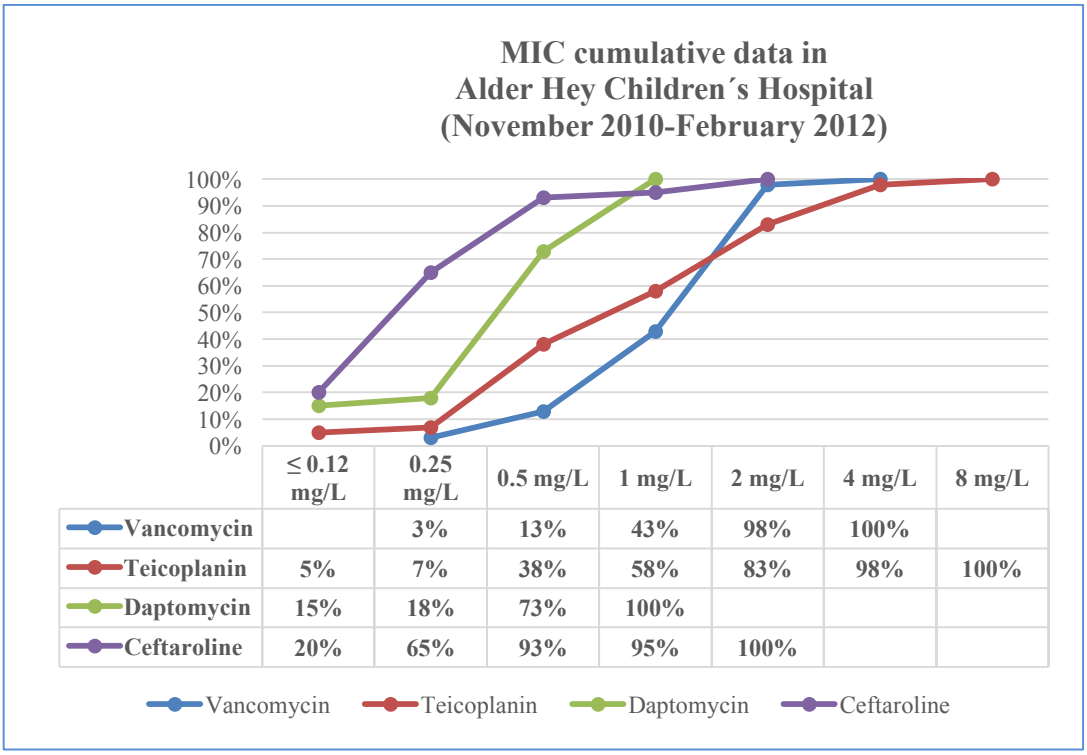
5.2- INTRODUCTION

Gram-positive bacterial infections are an important cause of morbidity and mortality in neonatal and paediatric intensive care units (Venkatesh et al. 2006b; Verstraete et al. 2014). A significant rise in infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (CoNS) has led to an increased use of glycopeptides in the last decade (Tiemersma et al. 2004; Van Den Hoogen et al. 2009). Both vancomycin and teicoplanin are used for treatment of invasive infections caused by Gram-positive organisms and especially those that are resistant to β-lactam antibiotics (Mermel et al. 2009; Gould et al. 2012). The currently recommended regimen for teicoplanin in adults is 3 loading doses of 400 mg 12 hourly, followed by a maintenance dose of 400 mg/day. In contrast, children receive 3 loading doses of 10 mg/kg 12 hourly, followed by a maintenance dose of 10 mg/kg q24h (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015b). Importantly, however, there is relative paucity of information to justify these regimens in children and even less information to identify optimal dosing strategies.

Teicoplanin is a glycopeptide antibiotic agent that has bactericidal activity against Gram positive aerobic and anaerobic bacteria (A. P. R. Wilson 2000). It is widely used for the treatment of invasive infections such as septicaemia, intravascular device-associated infections, endocarditis and septic arthritis caused by methicillin-resistant Gram-positive pathogens. Despite its extensive use, there are comparatively few pharmacokinetic-pharmacodynamic (PK/PD) data for teicoplanin compared with vancomycin. There is even less information on the PK of teicoplanin in neonates and children and evidence for currently recommended regimens is scant.

Regulatory authorities, such as the European Medicines Agency (EMA) have developed strategies to facilitate the safe and effective use of medicines in neonates and children (The European Parliament and The Council of the European Union 2006). The EMA supports the extrapolation of information from adults to children provided there are adequate safety data in the latter and the pharmacodynamics can reasonably assumed to be the same in both populations. This approach requires the development of robust population pharmacokinetic (PK) models in both adults and children, which in turn facilitates the design of regimens that enables drug exposures in both populations to be matched.

Teicoplanin is largely used without routinely measuring levels in the majority of paediatric patients. In our paediatric hospital setting, we have anecdotal evidence of clinical failures with teicoplanin therapy and have also observed "MIC creep" for CoNS often found at the breakpoint (4mg/L) (shown in **figure 5.0**).



	≤ 0.12 mg/L	0.25 mg/L	0.5 mg/L	1 mg/L	2 mg/L	4 mg/L	8 mg/L
Vancomycin		3%	13%	43%	98%	100%	
Teicoplanin	5%	7%	38%	58%	83%	98%	100%
Daptomycin	15%	18%	73%	100%			
Ceftaroline	20%	65%	93%	95%	100%		

Figure 5.0- MIC cumulative data from Alder Hey Children´s NHS Foundation Trust (Microbiology) corresponding to November 2010-February 2012. Data on 40 CoNS isolates recovered from paediatric blood cultures.

To further investigate the clinical pharmacology of teicoplanin and to provide an insight into effective regimens for children, we performed a population PK study. The specific objectives of this study were as follows: (1) to describe the population PK of teicoplanin in children in a hospital setting; (2) to explore the percentage of patients attaining a pre-dose concentration (C_{min}) > 10 mg/L; (3) define the area under the concentration-time curve (AUC) distributions following the administration of currently recommended paediatric regimens; and (4) compare the extent of variability in drug exposure to that observed in adults.

5.3- MATERIALS/PATIENTS AND METHODS

5.3.1- Study design, paediatric patient population and sample collection

An open label, hospital-based PK study using a sparse blood sampling strategy was conducted. Patients ≥1 month-<16 years of age were recruited from Alder Hey Children´s Hospital. The study was approved by the Medicines and Healthcare products Regulatory Agency (Clinical Trial Authorization number 21362/00003/001-0001) and the National Research Ethics Service and Regional Committee. The trial was registered with the European Clinical Trials Database Registry (EudraCT: 2012-005738-12).

Participants were screened and recruited according to three age-categories to ensure a representative sample was obtained. Such an approach is consistent with the European Medicines Agency E11 Guidance for Clinical Trials of Investigational Products (CTIMP) in paediatrics (European Medicines Agency 2011). The following categories were used 1-23 months, 2-11 years and 11-<16 years. A prospective feasibility assessment over 4 months conducted before the trial indicated that the pediatric intensive care unit, the oncology Unit, and the intermediate care units were the locations with a higher prescription rate of teicoplanin and therefore, these units were chosen for targeted screening and recruitment of participants. All patients that were prescribed teicoplanin and likely to survive more than 72 hours, were eligible for the study. Written informed consent was obtained from parents and/or legal guardians.

Teicoplanin was used at the discretion of the treating physician. The dosage regimen for children > 1 month of age was as follows: 10 mg/kg every 12 hours for 3 loading doses followed by 10 mg/kg once daily. Teicoplanin was infused over 5 minutes in children. The duration of treatment was also at the discretion of the treating physician.

Blood samples (0.2 mL) were obtained throughout the first and last dose interval (1, 3, 6 and 24h post-dose). If the first dose administration occurred before informed consent had been obtained, a pre-dose sample was also obtained. When possible, a washout sample was collected 24 hours after the last dose was administered. Samples were centrifuged at 1500 g for 10 minutes and serum was stored at -80º C prior to analysis. The following demographic variables with a potential impact on the PK of teicoplanin and/or influence the determination of teicoplanin (concomitant medications) were also collected for each patient: weight, height and serum creatinine.

5.3.2- Adult patient population

Adult patients with normal renal function who were previously treated with chemotherapy because of acute lymphocytic or acute non-lymphocytic leukaemia, and subsequently developing febrile neutropenia were recruited to a prospective observational PK study, as previously reported (Pea et al. 2004). All patients received teicoplanin for the first 72 hours. Subsequently, if a Gram-positive organism susceptible to teicoplanin was isolated and /or resolution of fever was documented within 72 hours, teicoplanin therapy was continued for at least 8 days. There were two dosing groups: firstly, the standard dosing group received standard loading and maintenance dosages of teicoplanin (400 mg every 12 hours for three doses followed by 400 mg once daily); secondly, the high dosage group received a higher loading regimen (800 followed by 400 mg, 12 hours apart on day 1; 600+400 mg 12 hours apart on day 2 followed by a high maintenance regimen (400 mg every 12 hours on day 3 and thereafter). Teicoplanin was infused over 15 minutes. Blood samples were collected at defined times: 1 h after the first dose to assess the peak level and at 12, 24, 48, 72, 96, 120 and

144 hours to estimate terminal elimination. *These adult PK data were kindly shared by Professor Federico Pea.*

Demographic data was analysed with SPSS Statistics version 21 (IBM Corporation, New York, United States, [http://www-01.ibm.com/software/analytics/spss/downloads.html]).

5.3.3- Teicoplanin Concentration Determination

A fluorescence polarization immunoassay (FPIA; Thermo Fisher Scientific, Germany) was used for the quantification of teicoplanin concentrations in serum. This is a homogeneous particle-enhanced turbidimetric immunoassay that utilizes the Quantitative Microsphere System (QMS) technology and was implemented on an automated analyser Abbott Architect ci4100. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody biding sites of the teicoplanin antibody reagent. A concentration-dependent agglutination inhibition curve was obtained with minimum and maximum rate of agglutination at the highest and lowest teicoplanin concentrations, respectively. The limit of quantification (LOQ) was < 3.0 mg/L. The dynamic range was 3-100 mg/L and total precision was < 6%. *The method was performed by Kevin Padmore in the AHFT's Clinical Pathology Laboratory.*

5.3.4- Population Pharmacokinetic Models

All data were analysed using a non-parametric population modelling methodology (Non-Parametric Adaptative Grid, NPAG) with the population pharmacokinetic software program Pmetrics (version 1.2.6. University of Southern California, California, United States, [http://www.lapk.org/pmetrics.php]) (Neely et al. 2012) for R (version 3.1.0, Institute for Statistics and Mathematics, Vienna, Austria, [http://www.r-project.org/](R Team 2013). The inverse of the estimated assay variance was used as the weighting function for all models.

Three structural models were explored and used in this study. The first represented a standard two-compartment PK model with time-delimited zero-order intravenous infusion and first-order elimination from central compartment. The model is described by the differential equations 1a and 1b below.

$$\frac{dX(1)}{dt} = R(1) - \left(Kcp + \frac{SCL}{Vc}\right) \cdot X(1) + [Kpc \cdot X(2)] \quad (1a)$$

$$\frac{dX(2)}{dt} = Kcp \cdot X(1) - Kpc \cdot X(2) \quad (1b)$$

Where X(1) and X(2) represent the amount of teicoplanin in milligrams (mg) in the central (c) and peripheral (p) compartments, respectively. R (1) is the rate of infusion of drug into the central compartment in mg per hour (mg/h). The central compartment has volume (Vc) in litres (L), from which there is clearance (SCL) in litres per hour (L/h). The central and peripheral compartments are connected by the first-order rate constants Kcp and Kpc (h^{-1}).

The effect of weight and serum creatinine on the population PK of teicoplanin was explored. The Bayesian estimates for clearance and volume of distribution from each patient were obtained from the standard model (above) and plotted against weight and serum creatinine, using both linear and logarithmic scales. Since both linear and logarithmic relationships between clearance and weight appeared tenable, linear and allometric models that incorporated weight as a covariate were developed. The linear model took the following form:

$$\frac{dX(1)}{dt} = R(1) - \left(Kcp + SCLslope \cdot \frac{weight}{Vc}\right) \cdot X(1) + [Kpc \cdot X(2)] \quad (2a)$$

$$\frac{dX(2)}{dt} = Kcp \cdot X(1) - Kpc \cdot X(2) \quad (2b)$$

Where SCL_{slope} represented the slope of the linear relationship between clearance and weight. The other terms and relationships are the same as were described for the standard model. The intercept of the linear relationship between clearance and weight was initially included in the structural model, but estimates from preliminary runs were approximately zero. Consequently, in the linear model SCL= SCL_{slope}*weight.

Since a relationship between the log_{10}-transformed estimates for weight and clearance from the standard model was also apparent, the performance of an allometric power model was also investigated. Such model has been used to determine the effect of size on the pharmacokinetics of various compounds in children and neonates (Wurthwein et al. 2005; Hope et al. 2007). The allometric scaling exponent in equation 3a was fixed at 0.75. In addition, only clearance and not volume appeared to have a relationship with weight, and therefore clearance was normalized to a 70 kg adult, as described elsewhere (Hope et al. 2007).

The differential equations describing the allometric model are as follows:

$$\frac{dX(1)}{dt} = R(1) - \left[Kcp + SCLstd \cdot \frac{\left(\frac{weight}{70}\right)^{0.75}}{Vc} \right] \cdot X(1) + [Kpc \cdot X(2)] \quad (3a)$$

$$\frac{dX(2)}{dt} = Kcp \cdot X(1) - Kpc \cdot X(2) \quad (3b)$$

Where SCLstd represents the normalized estimate for clearance in a 70 kg individual, the other parameters are described above.

For the adult data, a standard two-compartment structural model was used.

5.3.5- Model evaluation, comparison and performance

For each model, scatter plots of the observed-predicted relationships for each individual patient and the population as a whole were examined. Goodness-of-fit was evaluated on the basis of a visual inspection of the data, coefficient of determination of a linear regression of the observed-versus-predicted values in the scatter plot after the Bayesian step, as well as the slopes and intercepts of the regression. The log-likelihood values of each model were used to compare models. Statistical comparisons were made using the likelihood ratio test, where twice the likelihood difference was evaluated against a χ^2 distribution with the appropriate number of degrees of freedom. Predictive performance was based upon the weighted mean error and the bias-adjusted weighted mean squared error.

5.3.6- Monte Carlo Simulations

Monte Carlo simulations were performed using the linear model for children and the standard model for adults. The structural model was implemented within the simulation module of the pharmacokinetic program ADAPT 5 (W. X. D'Argenio DZ, Schumitzky A 2009). The covariance matrix was inserted into subroutine PRIOR.

Another subroutine within ADAPT 5 (courtesy of David D'Argenio, University of Southern California) enabled teicoplanin to be administered to each of 5,000 simulated patients on a weight basis (in mg per kg). For each simulated patient, the weight-based dose of teicoplanin (administered in mg per kg) was converted internally to an absolute dose of teicoplanin (in mg) by multiplying by the simulated weight. Thus, the simulation process mimicked drug administration as it occurred at the bedside in which the dose of teicoplanin was planned on an mg per kg basis, but the absolute amount of drug administered to each patient was determined with reference to weight. To ensure consistency with the clinical trial, teicoplanin was infused over 5 minutes to simulated children older than 1 month of age with fixed weights of 10, 25 and 50 kg. For adults, the same methodology was used applied to their dosage regimen and infusion time.

Both normal and log-normal parameter distributions were explored and discriminated on the basis of their ability to recapitulate the original parameter means and their dispersions. All calculations were performed at steady state between day 4 and day 5 post-initiation of treatment. The area under the concentration-time curve from time 96-120 h (AUC_{0-24}) was determined by integration and the proportion of patients achieving the desired drug exposure. A C_{min} >10 mg/L was used as the PK target throughout the study, as this has been suggested to be the current surrogate for efficacy for both, adults and children for most indications (Harding et al. 2000).

5.4- RESULTS

5.4.1- Demographics

A description of demographic data by the age categories is presented in **table 5.1.** A total of 39 patients recruited over 8 months (between April 2013 and December 2013), contributed 49 treatment episodes. Eight patients contributed to > 1 episode (six patients contributed two episodes, and two patients contributed three episodes, respectively). The mean age (standard deviation) was 4 (4.3) years. The mean weight at inclusion to the study (SD) was 17.27 kg (13.3), and 53.8% (n=21) were male. The mean height (SD) was 97.68 (34.76) cm, but was only recorded in 30 patients. Patients had a mean serum creatinine at start of treatment (SD) of 5.52 (50.08) μmol/L, only recorded in 32 patients.

The majority of patients had undergone cardiac surgery and was being nursed in the oncology unit (43.6%). The next largest population group was the patients being treated on the intensive care unit (33.3%). The remaining participants were being treated for general medical (20.5%) and cardiac medical (2.6%), in which teicoplanin is often used to treat catheter-associated bloodstream infections.

Thirty-three adult patients, 11 in the standard dosage group and 22 in the high dosing group were analyzed, 54.5% of them were male. Mean age (SD) was 47.2 (13.9) years. The mean weight (SD) was 67.7 (13.6) kg and the mean creatinine at the start of treatment (SD) was 69.41 (23.78) μmol/L. They all had been previously treated with chemotherapy because of acute lymphocytic or acute non-lymphocytic leukaemia, and subsequently developing febrile neutropenia.

Age Category	N (% total)	Age (years)	Weight (kg)	Height (cm)	Creatinine (µmol/L)
1-23 months	16 (41.02)	0.25 (0.45)	6.26 (2.04)	65.84* (11.93)	71.54 (75.68)
2-11 years	20 (51.3)	5.4 (2.23)	21.18 (7.14)	112.41** (14.36)	43.3 # (13.9)
11-16 years	3 (7.7)	14.7 (0.58)	49.97 (10.6)	166.9 (3.59)	55 (8.48)
Total	39 (100)	4 (4.3)	17.27 (13.3)	97.7*** (34.8)	54.5## (50)

Table 5.1- Demographics of patients according to age categories.

Data are expressed as means (SD)*n=11; ** n=16; ***n=30; #n=13, ##n=32

5.4.2- Pharmacokinetic data

A total of 306 pharmacokinetic (PK) serum samples were collected and 298 PK samples were included in the analysis (mean of 7.6 samples per patient) for the children. Eight observations were excluded from the analysis for the following reasons: in one patient, a single concentration obtained immediately at the end of 1 hour infusion was substantially higher than the usual peak observed with other patients, and was inconsistent with other observations from the same patient. On further investigation, this patient was found to have a single lumen line that was used for drug administration and sampling. The remaining seven observations were excluded because of incorrect or absent time records. Sampling time period was up to a maximum of 264 for some patients, and up to 144-168h for the majority of patients (**Figure 5.1**)

As seen in **figure 5.1**, the teicoplanin concentration-time profile for the 39 paediatric patients was highly variable. Overall, twenty-one patients (53.85%) had serum concentrations below 10 mg/L, the serum concentration frequently used to guide dosage adjustment.

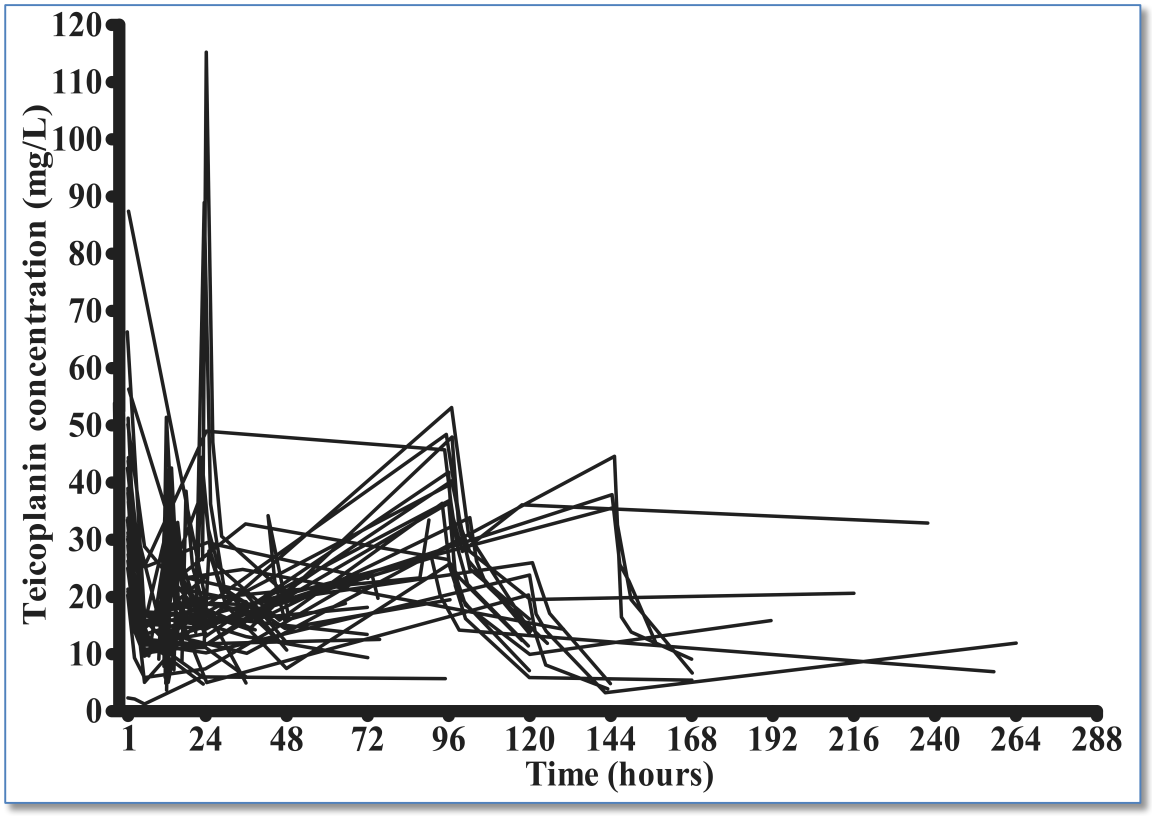


Figure 5.1- Teicoplanin serum concentrations in the 39 patients.

Patients were dosed according to recommended regimens. Children > 1month: 3 loading doses every 12 hours, then once daily.

5.4.3- Population Pharmacokinetic Models

The population parameter estimates from each of the models developed are summarized in **Table 5.2**. The relationship between the Bayesian estimates of clearance, obtained using the median population parameter values from the standard model and weight is shown in **Figure 5.2**. For the three models, the fit of the data was acceptable (r^2 0.742-0.814) with comparable measures of bias and precision. However, the better (more positive) log-likelihood value for both the linear and the allometric model, suggested that the inclusion of weight as a covariate enabled a significant

portion of the observed variance to be explained. The linear model was finally chosen to account for the observed data (r^2 0.79), shown in **Figure 5.3 (A, B)**. For adults, the data from both dosing groups (n=33) was examined using a standard two-compartment model, shown in **Figure 5.3 (B, C).** The model diagnostics are shown in **Table 5.3**.

The Bayesian estimates for the AUCs from each of the 39 paediatric patients are shown in **Figure 5.4**. The mean (SD) total population (n=39) AUC was 527.049 (166.035) mg*h/L.

	Mean	**Median**	**SD**
Clearance (L/h)	0.396	0.279	0.347
Vc (L)	4.259	2.592	3.597
Kcp(h^{-1})	3.344	0.434	7.742
Kpc(h^{-1})	4.424	0.252	9.742
Clearance slope (L/h/kg)	0.023	0.019	0.010
Vc (L)	4.138	2.282	4.143
Kcp(h^{-1})	3.876	0.474	8.156
Kpc(h^{-1})	3.994	0.292	8.930
Allometric			
Clearance Θ (L/h/ $kg^{0.75}$)	0.045	0.040	0.020
Vc (L)	3.447	1.975	3.579
Kcp(h^{-1})	4.897	0.564	9.054
Kpc(h^{-1})	4.227	0.228	9.638
Standard for adults			
Clearance (L/h)	1.166	1.097	0.376
Vc (L)	7.925	7.669	1.849
Kcp(h^{-1})	1.179	1.158	0.449
Kpc(h^{-1})	0.154	0.155	0.087

Table 5.2- Population Teicoplanin PK parameters estimated values in children and adults.

Clearance slope=Clearance /weight because Cl=Cl slope*wt; Clearance Θ = Clearance /[(weight/70) $^{0.75}$] because Cl=Cl Θ*(wt/70) $^{0.75}$; Vc= Volume of distribution in central compartment; Kcp= first-order constant rate from the central to peripheral compartment; Kpc= first-order constant rate from peripheral to central compartment.

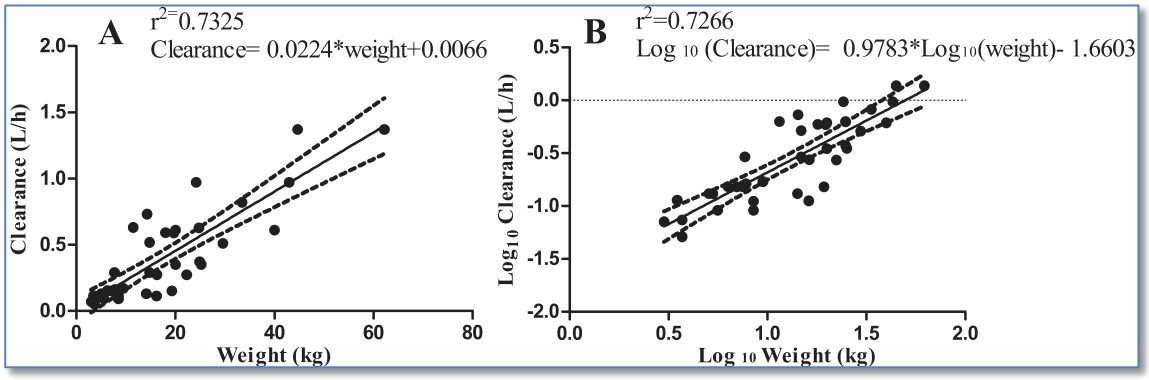


Figure 5.2- Evaluation of relationships between the Bayesian PK parameter individual estimates from the standard model for clearance and the weight of patients.

(A) Linear relationship between Bayesian Clearance estimates *versus* weight. (B) Linear relationship of the log-transformed values of Bayesian Clearance estimates *versus* weight. Dotted lines represent the 95% CI of the regression line.

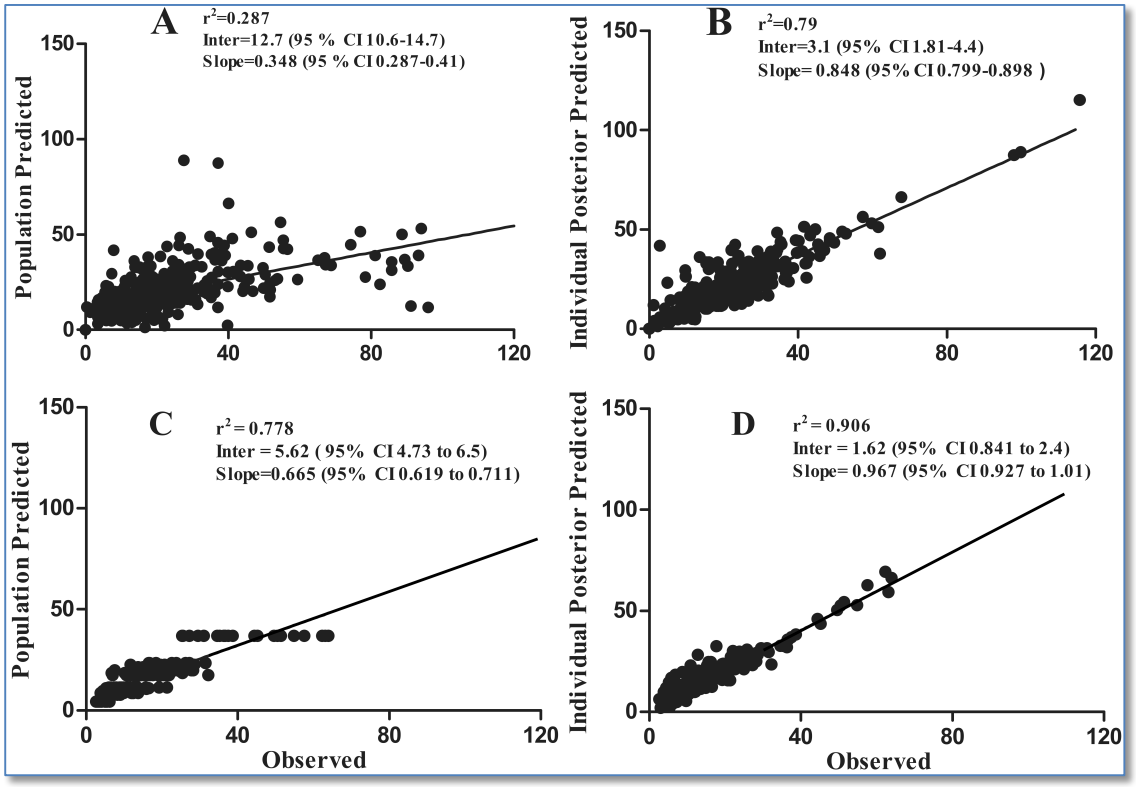


Figure 5.3- Observed *versus* predicted plots for the population and Bayesian posterior values in the linear model for children (A) and (B), respectively, and for the population and Bayesian posterior values in the standard model for adults (C) and (D).

	Log-likelihood	r^2 *	Slope (95%CI)*	Intercept (95%CI)*
Standard	-987.60	0.814	0.839 (0.793-0.884)	3.67- (2.49-4.85)
Linear	-979.212	0.79	0.848 (0.799-0.898)	3.1 (1.81-4.4-)
Allometric	-983.428	0.742	0.917 (0.857--0.978)	3.15 (1.68-4.61)
Standard - adults	-501.381	0.906	0.967 (0.927-1.01)	1.62 (0.841-2.4)

Table 5.3- Population PK model diagnostics for teicoplanin in children and adults.

***Relative to the regression line fitted for the observed versus predicted values after the Bayesian step.**

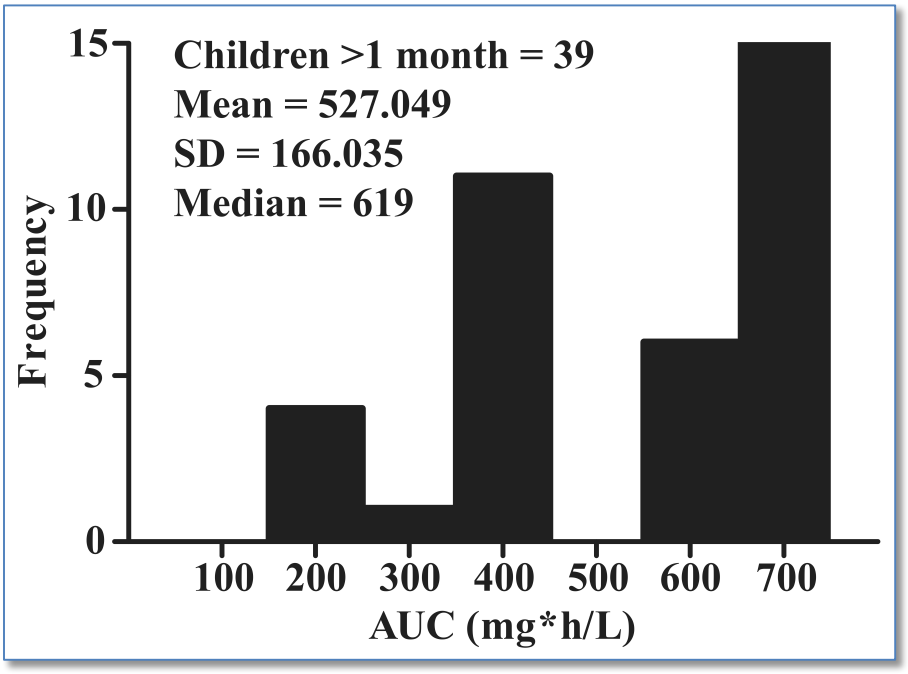


Figure 5.4- AUC distributions from the Bayesian posterior estimates from the linear PK model in children.

5.4.4- Monte Carlo Simulations

Monte Carlo Simulations were performed with the linear model in children (n=5,000). The extent of the predicted variability in serum teicoplanin concentrations and the resultant AUCs within a simulated human population receiving the current dosage regimen for children older than 1 month of age, at steady state (between days 4 and 5 of treatment) and for fixed weights 10 kg (1-23 months), 25 kg (2-11 years) and 50 kg (11-16 years) were obtained. Based on these simulations, only 30.04 % (median AUC_{0-24} = 507.04), 44.88 % (494.1) and 60.54% (452.03) of patients weighting 50, 25 and 10 kg respectively, attained trough concentrations >10 mg/L by day 4 of treatment. For adults, simulations (n=5,000) were performed with the standard model and current recommended regimen of three loading doses of 400 mg every 12 h and 400 mg once daily thereafter. Median AUC_{0-24} was 291.81mg*h/L, with 25 and 75 percentiles of 236.04 and 364.63, respectively. The simulated AUC distributions for children and adults are shown in **Figure 5.5.** A total of 24.8% of simulated adult patients attained trough concentrations >10 mg/L by 96 hours of treatment.

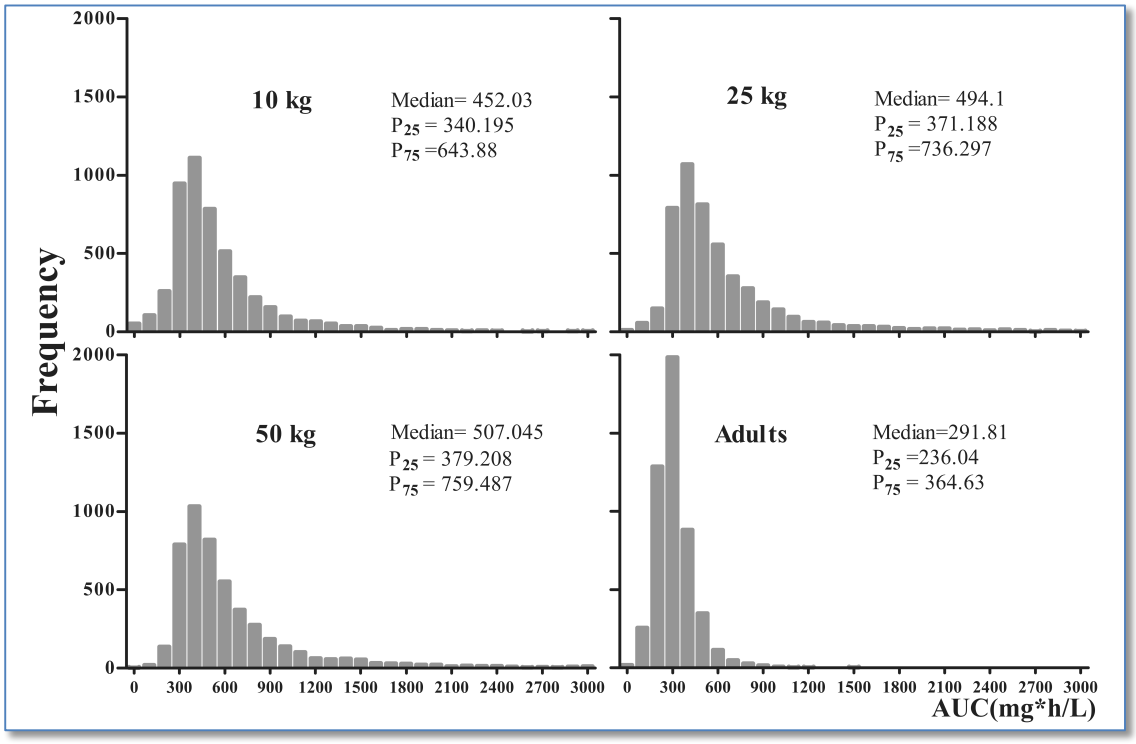


Figure 5.5- AUC distributions based on Monte Carlo simulations for children at fixed weights and for adults (not fixed weight).

Measures of medians, 25 and 75 percentiles (P_{25} and P_{75}, respectively)

5.5- DISCUSSION

We conducted a PK study in children and developed a population PK model to quantify inter-patient variability. We encountered a series of findings that can be used to improve the use of teicoplanin in children. Of greatest concern was the low proportion of patients attaining minimum serum concentrations (C_{min} >10 mg/L) at steady state with current recommended dosages. This is consistent with previous PK studies of teicoplanin in children (Dufort et al. 1996; Sánchez et al. 1999; Strenger et al. 2013). For example, Dufort *et al*. reported that 55.6% of febrile neutropenic patients did not achieve C_{min} >10 mg/L (Dufort et al. 1996). Similarly, Sánchez *et al*. revealed 89% of patients had low concentrations (C_{min}< 10 mg/L) in a study of critically ill children and infants (Sánchez et al. 1999). The mean estimate of clearance in our study (0.023 L/h/kg) was similar to those published by Dufort *et al*. (0.029 L/h/kg), and slightly lower than reported by Sánchez *et al* (0.045 L/kg/h). Our findings are also in agreement with those of a retrospective study showing that among 340 treatments with teicoplanin in paediatric patients of different ages (92 neonate/infant episodes, 69 toddler episodes, 62 school-age children episodes and 117 adolescent episodes) initial C_{min} at day 2 to 4 after 10–15 mg/kg every 12 h for three loading doses and every 24 h thereafter were < 10 mg/L in 14.1% of cases (Strenger et al. 2013).

Previous studies have investigated the impact of age on the pharmacokinetics of teicoplanin in children. In a population of 13 children (2-12 years), Terragna *et al* found no significant linear correlation between teicoplanin elimination half-life and age (Terragna et al. 1988). In contrast, Tarral and colleagues investigated two populations separately of children (6 children with a mean age of 7 years, and 4 neonates with a mean age of 8.5 days). They showed that, volume of distribution was higher in neonates than in children (0.6 L/kg *vs.* 0.54 L/kg) and clearance was higher in older children than

in neonates (0.028 *vs.* 0.016 L/h/ kg)(Tarral et al. 1988). Finally, Lukas *et al* conducted a population PK analysis in 20 infants and children (4 months-10 years) in the ICU, showing also differences amongst two identified sub-populations separated around 12 months of age. In younger infants (<12 months) a 92% of concentrations were at target minimum levels (10 mg/L) versus a 65% of concentrations in older children (≥12 months)(Lukas et al. 2004). Interestingly, in this study, linear and nonlinear models of CL and V with weight failed, despite weight showing a linear relationship with CL and V, but only in children under 12 months of age. Instead, weight scaling of CL and V was successful describing the PK behaviour in the studied population. The population split in the two age categories gave a successful model in the study by Lukas *et al*. Notably, only 4 patients under 12 months contributed to the model. We did not conduct a split analysis by age to avoid losing robustness of the predicted pharmacokinetic variability in the Monte Carlo simulations by decreasing sample sizes (Tam et al. 2006). In our study, by contrast, only the influence of weight related to clearance in a linear model, improved the description of our data across childhood. Volume of distribution, however, did not show a tenable relationship with weight nor with age.

In the present study, children had more variability in drug exposures (quantified as AUC distributions at steady state) compared with adults (figure 5.5). In this study adults had a mean AUC of approximately 300 mg*h/L, although a previous study reported a higher mean AUC of 550 mg*h/L (Van Bambeke et al. 2004). In our study, children across different weights achieved a median AUC of 619 mg*h/L, which compares with drug exposures reported in adults. The relatively higher paediatric PK variability supports the use of routine therapeutic drug monitoring (TDM) to enable active adjustment of teicoplanin dosages. Collectively, our results suggest that the current dosage regimen in children is probably adequate in terms of median drug

exposures attained (AUC at steady state), but TDM should be considered to minimize the number of patients with sub-optimal drug exposure. Such an approach could improve safety, efficacy and prevent the emergence of antimicrobial resistance although further studies are required to examine this issue.

There are a number of issues and challenges that have prevented the more extensive use of teicoplanin. The first relates to the composition and synthesis of the compound, which is a mixture of six related subcomponents (A2-1, A2-2, A2-3, A2-4, A2-5 and A3-1). The A3-1 component is the core glycopeptide that is common to all teicoplanin-like compounds (Bernareggi et al. 1992). A degree of concern has been expressed related to the composition of generic teicoplanin products, and its potential impact on pharmacodynamics. A second issue relates to problems measuring concentrations in clinical samples. A number of analytical methods have been used (e.g. *Bacillus subtilis* bioassay, solid phase enzyme receptor assay (SPERA), fluorescence polarization immunoassay (FPIA) and high performance liquid chromatography (HPLC). Importantly, the readout from these different modalities differs. Thus, the conclusions of PK/PD studies are dependent on the analytical method that has been used (McMullin et al. 1994). A third issue is a relative lack of knowledge of the pharmacodynamics of teicoplanin. A C_{min} > 10 mg/L has been associated with higher clinical rate cures compared with values of 5 mg/L (Harding et al. 2000). Higher troughs serum concentrations (e.g. 15-20 mg/L) have been recommended for the treatment of endocarditis caused by *S. aureus* (Wilson et al. 1994). A pre-clinical pharmacodynamic study suggests that a teicoplanin fC_{max}/MIC ratio of at least 2-3 is required for efficacy (J D Knudsen et al. 2000). Other small clinical studies have suggested target AUCs as high as 750 mg*h/L are required to eradicate or cure MRSA

infections (Kanazawa et al. 2011; Hagihara et al. 2012). There is an urgent need to further evaluate the pharmacodynamics of teicoplanin against staphylococcal infections.

This study has several limitations. Firstly, the study participants largely did not have renal impairment. Teicoplanin is eliminated via the kidneys with clearance of the unbound drug by glomerular filtration with minimal tubular reabsorption and renal secretion (A. P. R. Wilson 2000). The impact of reduced renal function on clearance and therefore dosing could not be assessed. Finally, we could not correlate drug exposure with clinical outcomes in this study.

In conclusion, the population PK of teicoplanin is highly variable in children. TDM should be considered a component of routine care, although further work is required to identify drug exposure targets. The population PK model can now be used to construct algorithms for individualized dosing as previously described (Hope et al. 2013; Felton et al. 2014).

Chapter 6

Pharmacokinetics and Pharmacodynamics of Teicoplanin in Neonates: Making better use of C-Reactive Protein to Deliver Individualized Therapy

6.1 ABSTRACT

Background: There is uncertainty about the optimal teicoplanin regimens for neonates. The study aim was to determine the population PK of teicoplanin in neonates, evaluate currently recommended regimens and explore the exposure-effect relationships.

Methods: An open label pharmacokinetic (PK) study was conducted. Neonates from 26-44 weeks post-menstrual age (PMA) were recruited (n=18). The teicoplanin regimen was 16 mg/ kg loading dose, followed by 8 mg/kg once daily. Therapeutic drug monitoring (TDM) and dose adjustment were not conducted. A standard 2-compartment PK model was developed, followed by models that incorporated weight. A pharmacokinetic-pharmacodynamic (PK-PD) model with C-reactive (CRP) serial measurements as the PD input was fitted to the data. Monte Carlo simulations (n=5000) were performed using Pmetrics. The AUCs at steady state and the proportion of patients achieving the recommended drug exposures (i.e C_{min} >15 mg/L) were determined.

Results: The PK allometric model best accounted for the observed data. The PK parameters medians were: Clearance=0.435*(weight /70) $^{0.75}$ (L/h), Volume 0.765 (L), Kcp 1.3 (h^{-1}), Kpc 0.629 (h^{-1}). The individual time-course of CRP was well described using the Bayesian posterior estimates for each patient. The simulated median AUC_{96-120} was 302.3 mg*h/L, median C_{min} at 120 h was 12.9 mg/L. A 38.8% of patients attained a C_{min} >15 mg/L by 120h.

Conclusion: Teicoplanin population PK is highly variable in neonates, weight being the best descriptor of PK variability. A low percentage of neonates were able to achieve C_{min} >15 mg/L. The routine use of TDM and improved knowledge on the PD of teicoplanin is required.

6.2- INTRODUCTION

Gram-positive bacterial pathogens are an important cause of nosocomial infection in neonates (Stoll et al. 2002). The risk factors include prematurity and the extensive use of central venous catheters. There is high attributable mortality and the potential for serious longer-term morbidity (Stoll et al. 2002; Marchant et al. 2013; Adams-Chapman & Stoll 2006). Teicoplanin is a glycopeptide antibiotic with activity against methicillin-resistant staphylococci (MRSA and CoNS) and has several potential advantages over vancomycin including better tolerability, lower risk of nephrotoxicity and improved ease of administration (Svetitsky et al. 2009; Cavalcanti et al. 2010). In older children, there is considerable pharmacokinetic (PK) variability in comparison with adults, with a much wider drug exposure (AUC) distribution among children (Ramos-Martin et al. 2014). Little is known about the PK of teicoplanin in neonates.

Serial concentrations of C-reactive protein (CRP) are a useful adjunct to the clinical assessment of neonates with acute infection (Philip & Mills 2000). The diagnosis of neonatal bloodstream infection and its subsequent management remains challenging. Clinical signs are nonspecific (Marchant et al. 2013). Blood cultures are notoriously insensitive and often only intermittently positive in this population because of the low yield of small blood volumes collected (Connell et al. 2007; Lutsar et al. 2014). A fall in CRP is reassuring evidence of response to antimicrobial therapy and CRP is frequently used to guide antimicrobial therapy in neonates with proven or suspected infection (Benitz et al. 1998; Ehl et al. 1997). Much of this decision-making has not been formalised using pharmacokinetic-pharmacodynamic (PK/PD) models or dosing algorithms.

In this study, we developed a population PK/PD mathematical model to describe the serum pharmacokinetics of teicoplanin in neonates with the pharmacodynamics

quantified in terms of circulating CRP concentrations. Our objectives were as follows: (1) describe and quantify measures of central tendency and inter-patient neonatal PK variability; (2) evaluate teicoplanin exposure in the neonatal population with currently recommended regimens; and (3) investigate the relationship between drug exposure and the time-course of CRP. The latter is a first critical step for the development of algorithms to control both serum drug concentrations and clinically relevant biomarkers such as CRP.

6.3- MATERIALS/PATIENTS AND METHODS

6.3.1- Study design, patient population and sample collection

An open label, hospital-based PK study using a sparse blood sampling strategy was conducted. Both pre-term and term neonates from 26 to 44 weeks postmenstrual age (PMA) were recruited from Alder Hey Children´s NHS Foundation Trust and Liverpool Women´s NHS Foundation Trust (Liverpool, United Kingdom).

Participants were screened and recruited according to five age categories to ensure a range of gestations was studied. The following categories were used for recruitment: 24-27, 28-31, 32-35, 36-39 and 40-44 weeks PMA. All patients that received teicoplanin for proven or suspected CoNS sepsis and/or central-line associated infection and likely to survive more than 72 hours were eligible for the study. All patients also received ciprofloxacin or gentamicin as part of the combined empirical therapy for central-line associated bloodstream infection. Additional therapeutic interventions were: the use of inotropes, diuretics, paracetamol, anticonvulsants, muscle relaxants and assisted mechanical ventilation depending on the individual case.

Teicoplanin was used at the discretion of the treating neonatologist. The regimen for neonates ≤ 44 weeks PMA was a loading dose of 16 mg/kg, followed 24 hours later by 8 mg/kg administered once daily (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015a). Teicoplanin was infused over 30 minutes. Therapeutic drug monitoring (TDM) is not routinely performed and dose adjustment was not conducted in this study. The duration of treatment was also at the discretion of the treating neonatologist.

Blood samples (0.2 mL) were obtained throughout the first and last dose intervals (1, 3, 6 and 24h post-dose). Patients weighing <1000 grams at inclusion could

have a maximum of two study-specific sampling episodes per dose interval to minimise blood loss. The total sampling period was to a maximum of 168 h for the majority of patients. If the first dose administration occurred before informed consent had been obtained, a pre-dose sample was obtained. Whenever possible, a washout sample was collected 24 hours after the last dose. Samples were centrifuged at 1500 g for 10 minutes and serum was stored at -80ºC prior to analysis.

The demographic variables with a potential impact on the PK of teicoplanin and/or influence on the determination of teicoplanin (concomitant medications) were collected for each patient (i.e. weight and serum creatinine). Concentrations of CRP before, during and after teicoplanin treatment were measured as part of standard care. Demographic data were analysed with SPSS Statistics version 22 (IBM Corporation, New York, United States, [http://www-01.ibm.com/software/analytics/spss/downloads.html]).

6.3.2- Ethics

The study was approved by the Medicines and Healthcare Products Regulatory Agency (clinical trial authorisation reference number: 21362/0003/001-0002) and the National Research Ethics Service and Regional Committee (REC: 13/NW/0023). Written informed consent was obtained from parents and/or legal guardians. The study was registered in the European Clinical Trials Database Registry (EudraCT): 2012-005738-12.

6.3.3- Teicoplanin Concentration Determination

Teicoplanin concentrations were measured using a commercially available fluorescence polarization immunoassay (FPIA; Thermo Fisher Scientific, Germany)

The limit of quantification (LOQ) was < 3.0 mg/L. The dynamic range was 3-100 mg/L and overall precision was < 6%. *This method was performed by Kevin Padmore.*

6.3.4- Measurement of CRP Concentrations

A Multigen CRP Vario® (Abbott, Wiesbaden, Germany) latex immunoassay was used for the immunoturbidimetric determination of CRP in the plasma of patients, and also implemented in the Abbott Architect ci4100 system. The limit of quantification (LOQ) was < 0.2 mg/L (reported clinically as < 4 mg/L) for the standard and wide range methods (analyte concentration at which the CV=20%). The dynamic range was 0.2-480 mg/L (wide range method) and total precision was ≤ 6%. A CRP cut-off value > 10 mg/L was considered positive. *This method was performed by the Clinical Pathology Laboratory in AHFT as per standard of care.*

6.3.5- Microbiological Investigations

Microbiological specimens, including blood cultures, were collected as part of routine clinical care. Positive microbiological samples were stored for identification with a Bruker Biotyper MALDI-TOF MS System (Bruker Daltonics, Billerica, MA) and susceptibility testing was performed using E-test® (bioMérieux, Hampshire, UK), following BSAC methodology (British Society of Antimicrobial Chemotherapy 2006).

6.3.6- Population Pharmacokinetic Models

All data were analysed using Pmetrics (Neely et al. 2012). The inverse of the estimated assay variance was used as the weighting function for all models. Three structural PK models were explored in this study. The first represented a standard two-compartment PK model with time-delimited zero-order intravenous input and first-order

elimination from central compartment. The model is described by the differential equations 1a and 1b below.

$$\frac{dX(1)}{dt} = R(1) - \left(Kcp + \frac{SCL}{Vc}\right) \cdot X(1) + [Kpc \cdot X(2)] \quad (1a)$$

$$\frac{dX(2)}{dt} = Kcp \cdot X(1) - Kpc \cdot X(2) \quad (1b)$$

Where X(1) and X(2) represent the amount of teicoplanin (mg) in the central (c) and peripheral (p) compartments, respectively. R(1) is the rate of infusion of drug into the central compartment (mg/h). The central compartment has volume (Vc) in liters (L), from which there is clearance (SCL) in liters per hour (L/h). The central and peripheral compartments are connected by the first-order rate constants Kcp and Kpc (h^{-1}).

The effect of weight, PMA, post-natal age (PNA), serum creatinine and estimated glomerular filtration rate (eGFR) using the Haycock-Schwartz formula (K*Height/ serum creatinine)(Martini et al. 2003)(the UK population median height values for age and sex were used for each patient; and K=0.33 for pre-term neonates and K=0.45 for term neonates) on the population PK of teicoplanin was explored (Brion et al. 1986; Schwartz et al. 1984). The Bayesian estimates for clearance and volume of distribution from each patient were obtained from the standard model (above) and plotted against weight, PMA, PNA, serum creatinine and eGFR, using both linear and logarithmic scales. Since both linear and logarithmic relationships between clearance and weight appeared tenable, linear and allometric models that incorporated weight as a covariate were developed. Ultimately, an allometric power model was used. Such a model has been widely used to determine the effect of size on the pharmacokinetics of various compounds in children and neonates (Wurthwein et al. 2005; Hope et al. 2007). The allometric scaling exponent in equation 3a was fixed at 0.75. Only clearance and

not volume had a relationship with weight, and therefore clearance was normalized to a 70 kg adult, as described elsewhere (Hope et al. 2007). The differential equations describing the allometric model are as follows:

$$\frac{dX(1)}{dt} = R(1) - \left[Kcp + SCLstd \cdot \frac{\left(\frac{weight}{70}\right)^{0.75}}{Vc} \right] \cdot X(1) + [Kpc \cdot X(2)] \quad (3a)$$

$$\frac{dX(2)}{dt} = Kcp \cdot X(1) - Kpc \cdot X(2) \quad (3b)$$

Where SCLstd represents the normalized estimate for clearance in a 70 kg individual; the other parameters are described above.

After establishing the model that best described the pharmacokinetics of teicoplanin, the following pharmacodynamic equation was used to describe the time-course of CRP concentrations:

$$\frac{dX(3)}{dt} = (KCRPprod * X(3) * \left(1 - \frac{X(3)}{POPmax}\right)) - (KCRPinh * X(3) * (\left(\frac{X(1)}{Vc}\right)^{H}))/((EC50^{H}) + (\left(\frac{X(1)}{Vc}\right)^{H})) \quad (3c)$$

Where KCRPprod is the maximum rate of CRP production (mg/L*h^{-1}), POPmax is the theoretical maximum CRP concentration (mg/L), KCRPinh is the maximum rate of CRP inhibition (mg/L*h^{-1}) induced by the drug, EC50 is the concentration of teicoplanin (mg/L) that produces half-maximal effect (CRP inhibition) and H is the slope function for the CRP inhibition term.

Given the high PK variability in the population and in order to avoid biased parameter estimates in the PK/PD model, the Bayesian posterior estimates for each patient´s PK parameters (from the final PK model described above) were fixed and the

PD parameters were then estimated by fitting the PD component of the model to each patient´s CRP data. The Bayesian posterior estimates for each subject were used to estimate the concentration-time profiles for teicoplanin and CRP for each patient. Average AUC and trough (C_{min}) for each 24 h of therapy were calculated from the Bayesian posterior estimates.

6.3.7- Monte Carlo Simulations

Monte Carlo simulations were performed using a semi-parametric sampling methodology that generated a simulated population of 5,000 neonates receiving a given teicoplanin regimen (Neely et al. 2012; Goutelle et al. 2009). For each simulated patient, the weight-based dose of teicoplanin (administered in mg per kg) was administered to each neonate (30-minute infusion) as an absolute dose of teicoplanin (in mg) by multiplying the rate of infusion (in mg/h) by the simulated weight.

All calculations were performed at steady state between day 4 and 5 of treatment. The proportion of patients achieving C_{min} >10, >15, 20, 30, 40 and 60 mg/L (the latter as the recommended safety cut-off) was determined. A comparison of the variability of distribution of drug exposures (AUCs) achieved in the neonatal simulated population was performed with the distribution of drug exposures in older children (1 month-16 years old) and adults receiving also currently recommended dosage regimens of teicoplanin. This comparison was based on Monte Carlo simulations conducted on a previous teicoplanin PK study with older children and adults (Ramos-Martin et al. 2014).

6.3.8- Exposure-response relationships

A newly described PD index (AUC: EC50) was used to link drug exposure with therapeutic response (terminal CRP concentration)(Huurneman et al. 2016). The

average $AUC_{0\text{-}24}$ was calculated from the Bayesian posterior estimates, divided by the median EC50 and the PD index plotted against the CRP Bayesian estimated values at the end of therapy. A sigmoid Emax inhibitory model was fitted to the data using the following equation:

$$Y = Baseline - (Emax * \frac{X^H}{X^H + EC50^H})$$

Where X is AUC:EC50, H is the Hill coefficient and EC50 is the median Bayesian estimate for the teicoplanin concentration that induces half maximal CRP reduction.

The use of a more conventional index (e.g. AUC:MIC ratio) was infeasible because the MIC of the invading microorganism was not available for the majority of patients. The EC50 is the estimated drug concentration required to induce half-maximal reduction in the CRP concentrations and it is therefore an *in vivo* estimate of drug activity.

6.4- RESULTS

6.4.1- Demographics

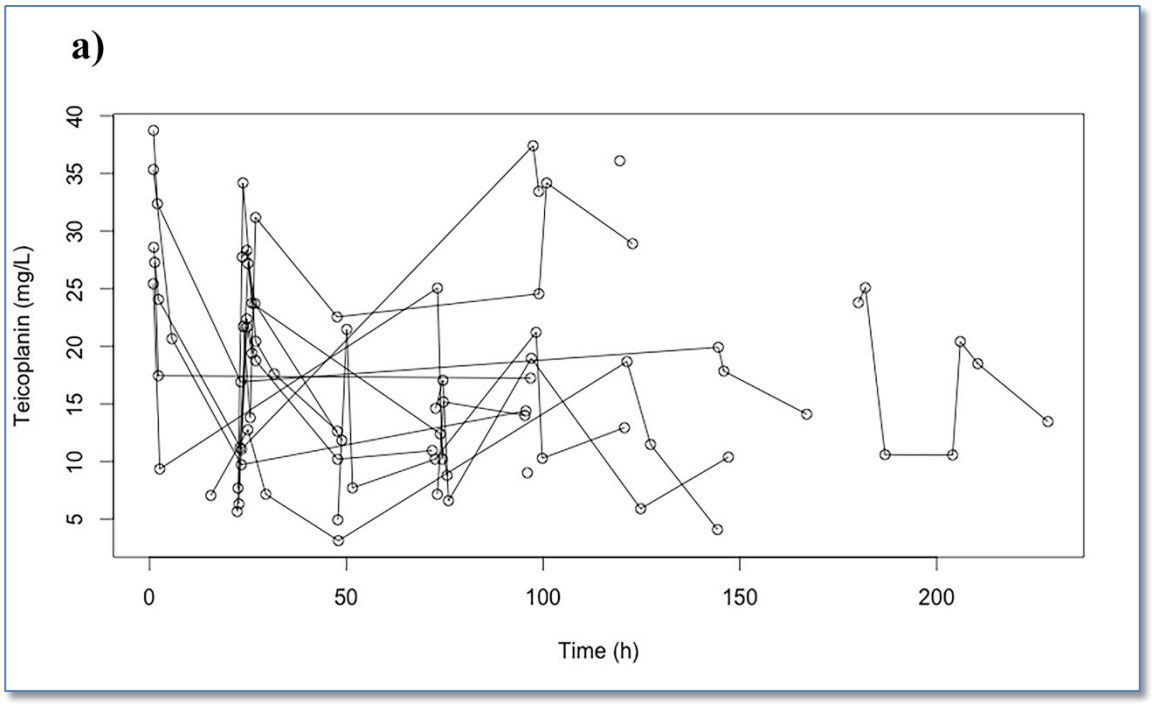
A total of 18 neonates were recruited from two different hospitals in Liverpool (Alder Hey NHS Children´s Foundation Trust and Liverpool Women´s Hospital) over a 20 month-period (April 2013 and January 2015). Ten patients were recruited from the neonatal intensive care unit at the Liverpool Women´s Hospital. The number of recruited patients by sub-category PMA (weeks) was: 24-27 (n=1), 28-31 (n=5), 32-35 (n=2), 36-39 (n=5) and 40-44 (n=5). A description of the demographic data is presented in **table 6.1.**

	n	Range	Median (IQR)
PMA (weeks)	18	26-44	37 (29.7-40)
Age (days)	18	4-69	17 (10.5-26)
Weight at birth (kg)	18	0.69-4.2	1.42 (0.9-3)
Weight at enrolment (kg)	18	0.69-5.08	2.04 (1.16-3.23)
eGFR (ml/min/1.73 m^2)	18	5.4-95.2	42.8(27.8-53.3)
Creat. (1st day of TEC) (µmol/L)	18	21-265	44.5 (36-57.7)
Creat. (last day of TEC) (µmol/L)	13	28-114	38 (34-63.5)
CRP (1st day of TEC) (mg/L)	18	4-172.6	41.9 (12.1-122.25)
CRP (last day of TEC) (mg/L)	16	4-163.7	6.9 (4-13.1)
PMA: Post-menstrual age. eGFR= estimated glomerular filtration rate (Schwartz-Haycock). Creat.= creatinine . TEC= teicoplanin. CRP = C-reactive protein. IQR= interquartile range.			

Table 6.1- Demographics of neonates included in the PK/PD teicoplanin study.

6.4.2- Teicoplanin and CRP Concentrations

The concentration time-profile of teicoplanin and corresponding CRP concentrations for each patient is shown in **figure 6.1a and 6.1b**, respectively. A total of 96 PK samples were available for analysis (mean of 5.3 samples per patient). Fourteen PK concentrations, from 4 patients were excluded from the analysis because of incorrect or absent sampling times. The mean (SD) from the observed teicoplanin concentrations was 18 (9.11) mg/L and a median of 17.32 mg/L (range 3.1-38.7 mg/L). A total of 104 CRP samples were available for analysis as part of the standard care of the patients.



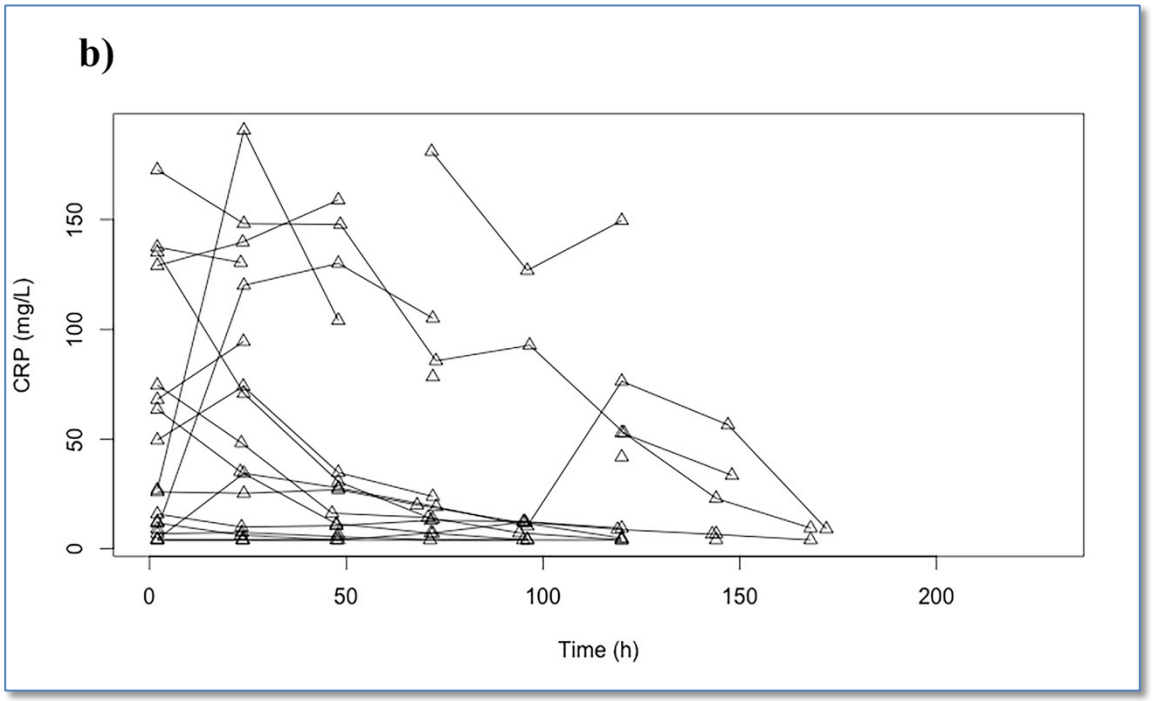


Figure 6.1- Teicoplanin and CRP concomitant concentration-time profiles from the neonates.

a) Teicoplanin (empty circles) and b) CRP (empty triangles) concomitant concentration time-profiles

6.4.3- Microbiological Results

A total of 44.4% of recruited patients (n=8) had a positive blood culture obtained from either a central or peripheral i.v. line. A total of 33.3% of those recruited (n=6) were Gram-positive infections (100% CoNS including *S. haemolyticus* (n=2), *S. epidermidis* (n=1) and n=3 unidentified species). All were susceptible to teicoplanin (MIC≤ 4 mg/L using the EUCAST clinical breakpoint)(European Committee on Antimicrobial Susceptibility Testing 2015). The remaining 11.1 % (n=2) were Gram-negative bacterial infections (n=1 had *P. aeruginosa* and n=1 had *Klebsiella oxytoca*) (These two patients only received two doses of teicoplanin each and were excluded from the subsequent exposure-relationship analysis).

6.4.4- Population Pharmacokinetic models

Both the population PK linear and allometric models performed similarly with an acceptable fit to the observed data and comparable measures of bias and precision. However, on the basis of the individual Bayesian estimates of the observed-*versus*-predicted fit of the data, the allometric PK model better accounted for the observed data and was chosen for further analyses. The model diagnostics are shown in **table 6.2.** For the allometric model the linear regression of observed *versus* predicted values had a coefficient of determination of r^2 = 0.815 with measures of bias and precision of 0.03 and 0.8, respectively (shown in **figure 6.2a**). The population PK parameter estimates of the allometric model are shown in **table 6.3.**

Model	Log-likelihood	Pop r^{2*}	Post r^{2*}	Slope (95% CI)	Intercept (95% CI)
Standard PK	-253.8	0.159	0.814	0.925 (-1.3-2.8)	0.768 (-1.3-2.8)
Linear (weight) PK	-254.1	0.271	0.807	0.941 (0.8-1.04)	0.922 (-1.14-2.98)
Allometric (weight) PK	-254.4	0.249	0.815	0.981 (0.9-1.1)	0.26 (-1.8-2.3)

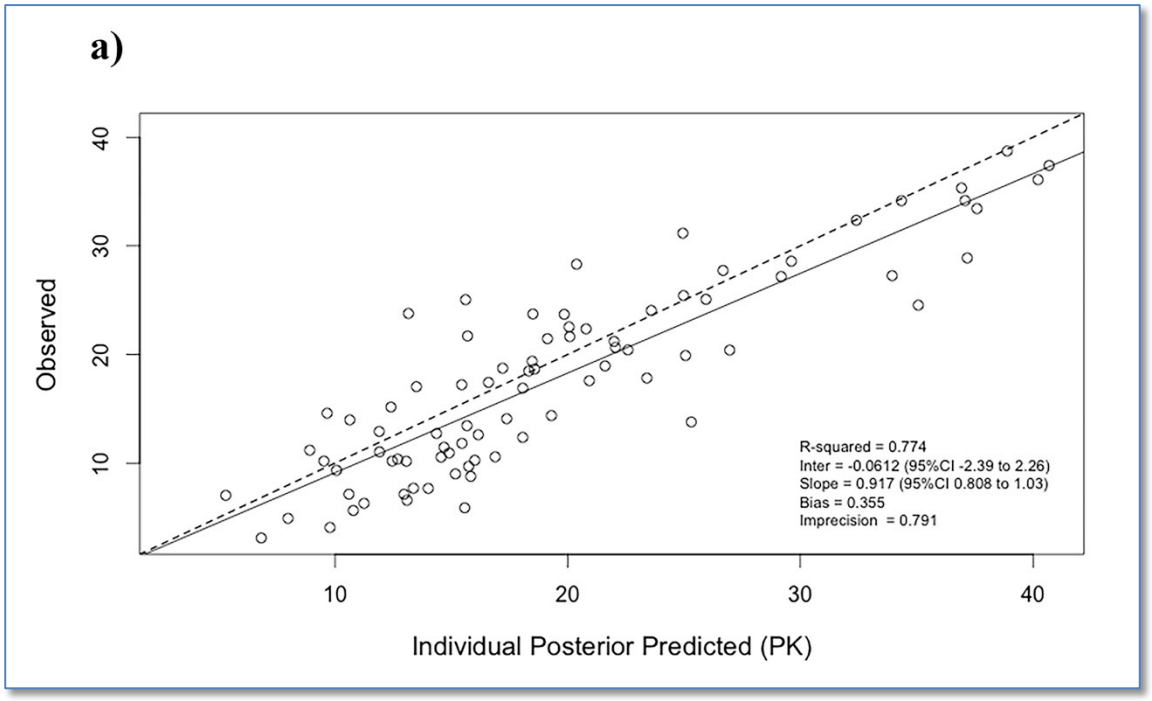
Table 6.2- Model diagnostics for the Population Teicoplanin PK models in neonates.

*** relative to the regression line fitted for the observed versus predicted values after the Bayesian step. CI: confidence interval.**

Population PK parameter	Mean	SD	Median
Clstd (L/h)	0.45	0.2	0.43
Vc (L)	0.81	0.48	0.76
Kcp (h^{-1})	1.45	0.99	1.3
Kpc (h^{-1})	0.84	1.05	0.63
Population PD parameter			
Kgmax ($mg/L*h^{-1}$)	0.05	0.03	0.05
Popmax (mg/L)	159.76	62.6	139.15
H	18.48	3.46	19.99
Kkmax ($mg/L*h^{-1}$)	0.05	0.02	0.06
EC50 (mg/L)	7.1	6.11	5.79
IC3 (mg/L)	55.32	54.24	24.99

Table 6.3- Population PK/PD parameter estimates for the allometric model in neonates.

Clstd= Clearance standardized from [Clearance=Clstd*$(wt/70)^{0.75}$]; Vc=Volume of distribution in the central compartment; Kcp and Kpc= firs-order rate constants from central to peripheral compartments and from peripheral to central compartments, respectively; Kgmax= maximum rate of CRP production; Popmax= theoretical maximum CRP concentration; H=Hill slope; C50= Teicoplanin concentration producing half-maximal CRP reduction; IC3= initial condition in CRP concentrations.



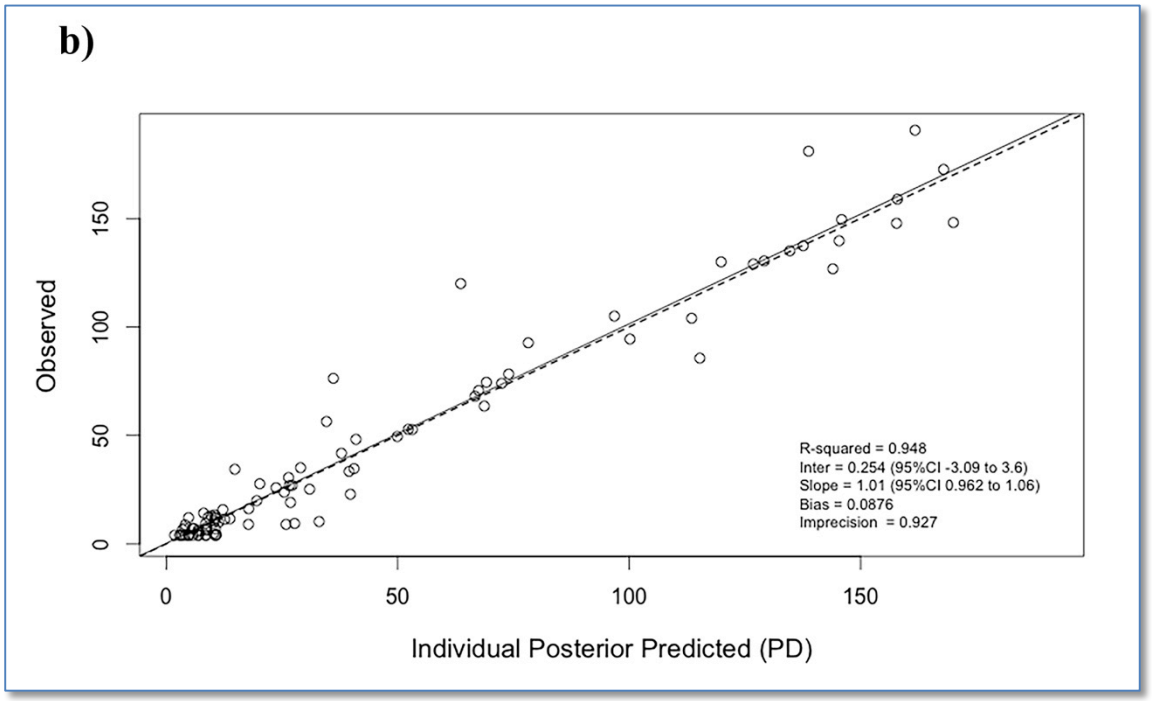


Figure 6.2- Individual posterior observed versus predicted plots (after the Bayesian step) from the PK/PD model.

Panel a) PK (teicoplanin concentrations), Predicted teicoplanin concentrations=0.917x-0.06; Panel b) PD (CRP concentrations), Predicted CRP concentrations= 1.01x+0.254

6.4.5- Population PK/PD Model

The fit of the PK/PD data was acceptable. The linear regression of observed *versus* predicted values had a coefficient of determination of $r^2 = 0.95$ with measures of bias and precision of 0.09 and 0.9, respectively (shown in **figure 6.2b**). The time-course of CRP in each individual patient was described with a high degree of precision and minimal bias using the Bayesian posterior median estimates for each patient. The population PK/PD parameter estimates are summarised in **table 6.3**. The Bayesian individual posterior estimates for the linked PK and the PD are shown in **Figure 6.3**.

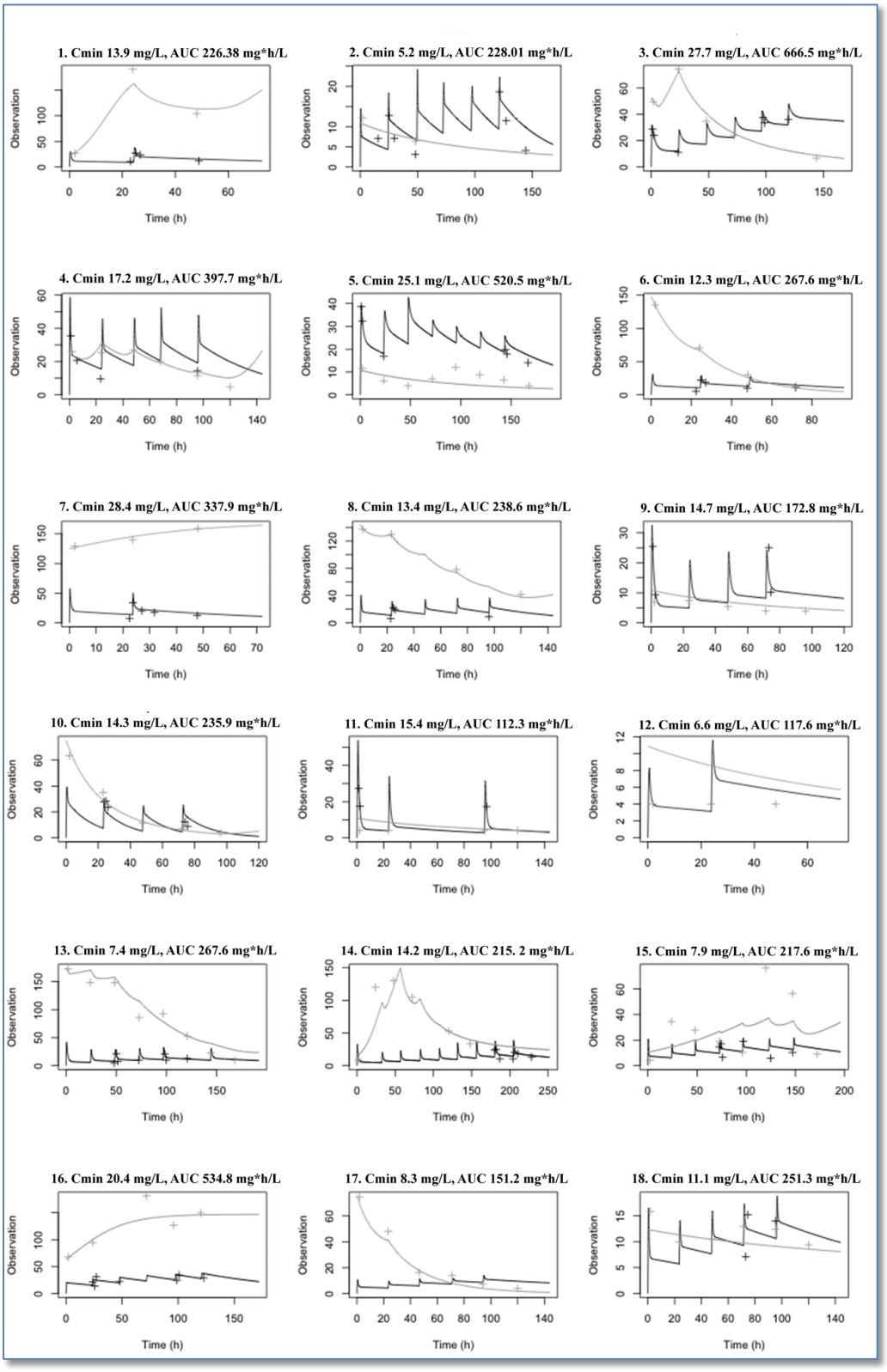


Figure 6.3- Individual concentration-time plots after the Bayesian step showing teicoplanin (black) and CRP (grey) concentrations. Predicted (continuous line) and observed (crosses) concentrations over time for each of the patients. The y axis "observations" refer to both: teicoplanin and CRP concentrations. Individuals 1 and 7 were infected with Gram-negative bacteria and only received 2 doses of teicoplanin. The individual average C_{min} and AUC drug exposures are reported for each patient.

6.4.6- Monte Carlo Simulations

Based on the simulations, the mean (SD) 24-hour steady-state AUC from 96-120 h was 365.4 (267.1) with a median of 302.3 mg*h/L. The mean (SD) trough at 96 h was 15.7 (11.7) mg/L with a median of 12.9 mg/L. Only 38.8 % of neonates achieved a C_{min} at 120h > 15 mg/L. In addition, 69.1%, 22.4%, 8.56%, 3.92% and 1.1% achieved C_{min} > 10, 20, 30, 40 and 60 mg/L, respectively. Comparative distribution histograms of the achieved AUCs at steady state for the simulated neonates, as well as for simulated older children and adult populations are shown in **figure 6.4**. The neonatal population achieved median AUCs at steady state (302.3 mg*h/L) comparable to the median AUC attained by a population of adults receiving 400 mg/day (291.81 mg*h/L), but with more variability (neonatal AUC IQR= 227.5 *versus* adult AUC IQR= 101.59 mg*h/L).

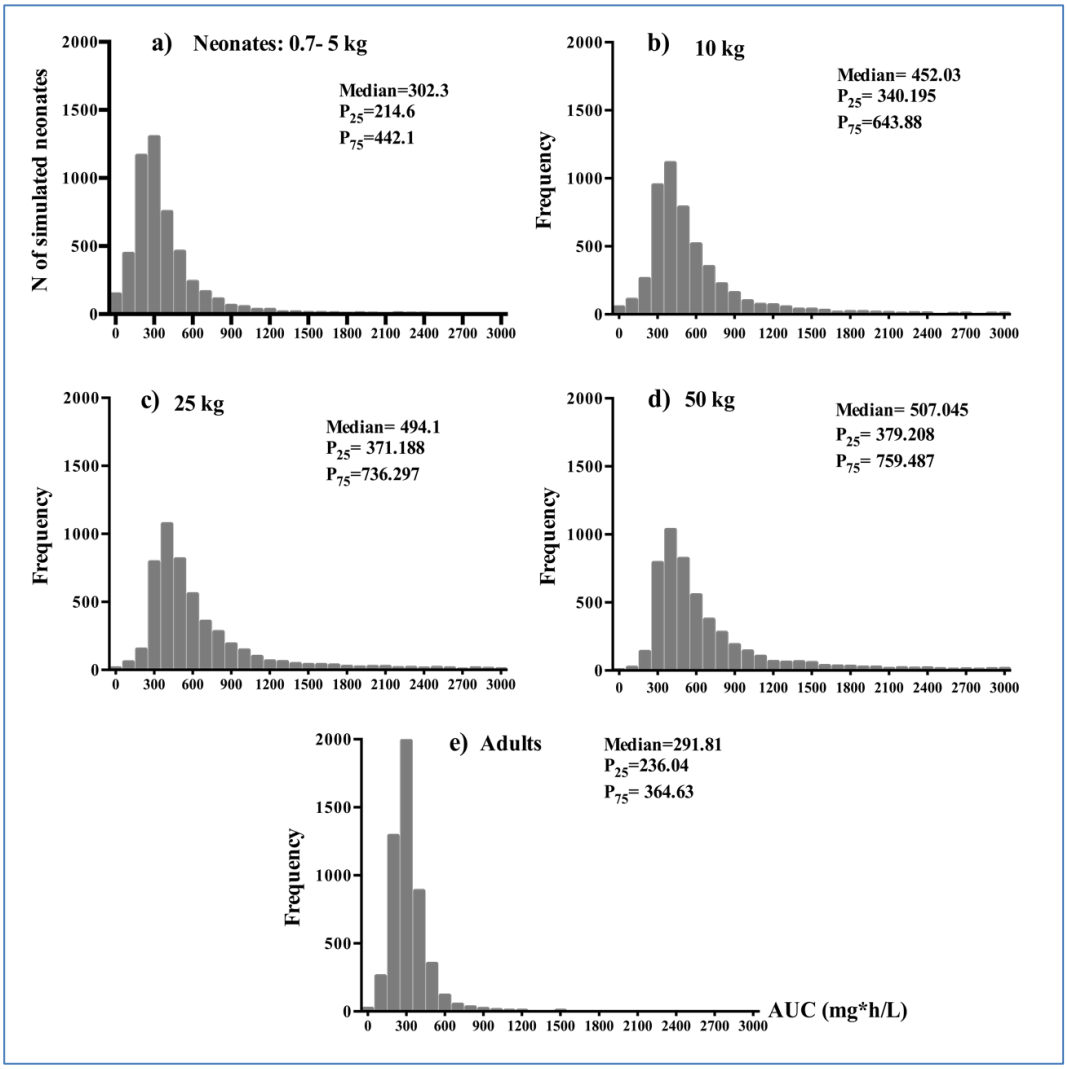


Figure 6.4- Comparison of simulated (n=5000 per population group) teicoplanin AUC (mg*h/L) distribution histograms in different populations.

a) neonates (0.7-5 kg) b), c) and d) children >1 month-16 years old with fix weights of 10, 25 and 50 kg, respectively and e) adults receiving current teicoplanin dosage regimen.

6.4.7- Exposure-response relationships

The Bayesian posterior estimates for the exposure-response relationships (AUC, C_{min} and AUC:EC50) are shown in **figure 6.5.** If patients 1 and 7 (infected with *Pseudomonas aeruginosa* and *Klebsiella oxytoca*, respectively) are excluded, 56 % of the patients (9/16) were able to suppress CRP under the cut-off value of 10 mg/L by 96-120 h. Subject 16 (gastroschisis) was not included in the inhibitory sigmoid Emax model (the patient's data are shown in **figure 6.5c**). An AUC:EC50 of ≈68.3 is predictive of a terminal CRP ≤ 10 mg/L. The relationship between AUC:EC50 and predicted CRP at the end of therapy is shown in **figure 6.5c.** Patients with an AUC:EC50 >68.3 tended to have a more consistently lower terminal CRP level than patients with an AUC:C50<68.3 (p=0.002) **(figure 6.6).**

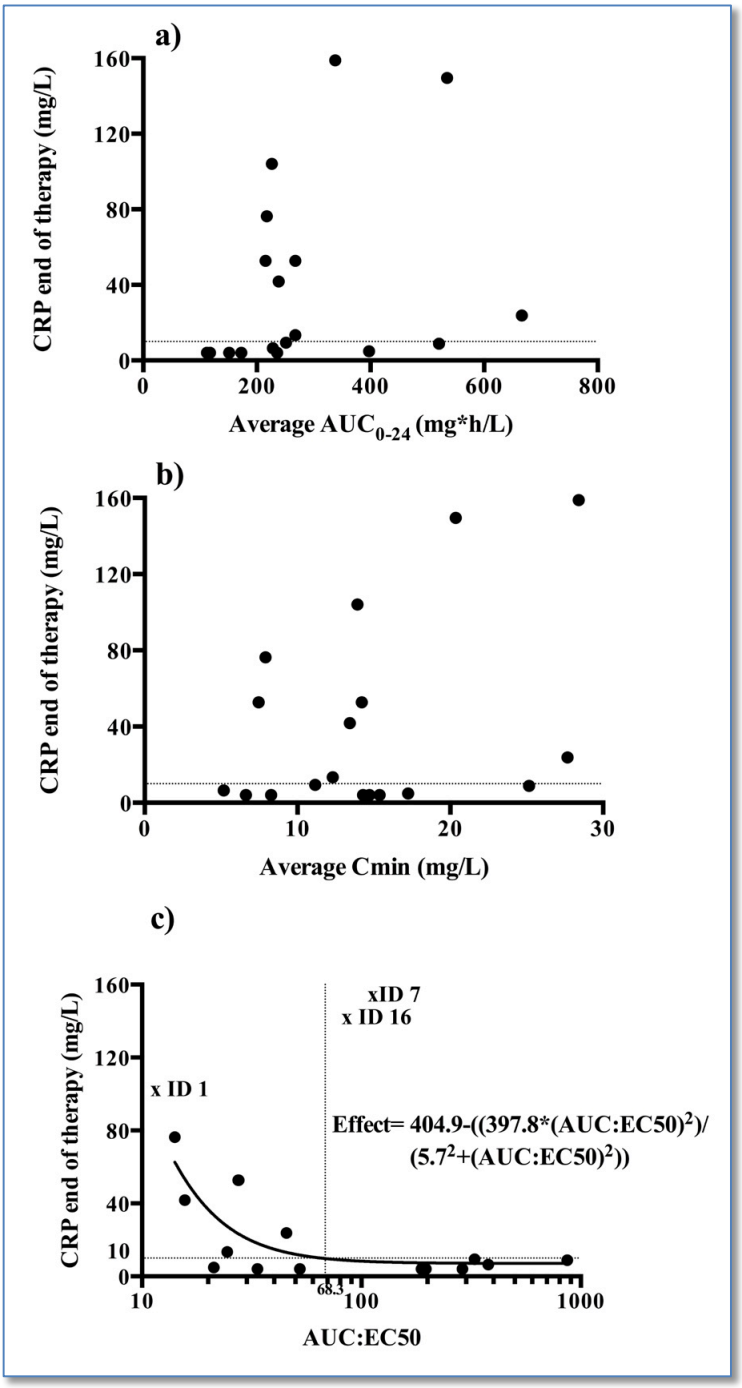


Figure 6.5- Exposure-response relationships from the Bayesian posteriors from the PK/PD linked model.

a) AUC average, b) C_{min} average and c) AUC:EC50 ratio (log_{10} scale) as the pharmacodynamic relevant index *versus* predicted CRP concentrations at the end of therapy. A sigmoid Emax inhibitory model was fitted to the data. Patients with ID 1, 7 (Gram-negative bacterial infection) and 16 (multiple inflammatory co-morbidities with persistently high CRP levels> 100 mg/L and negative blood culture) were excluded from this analysis but shown in panel c.

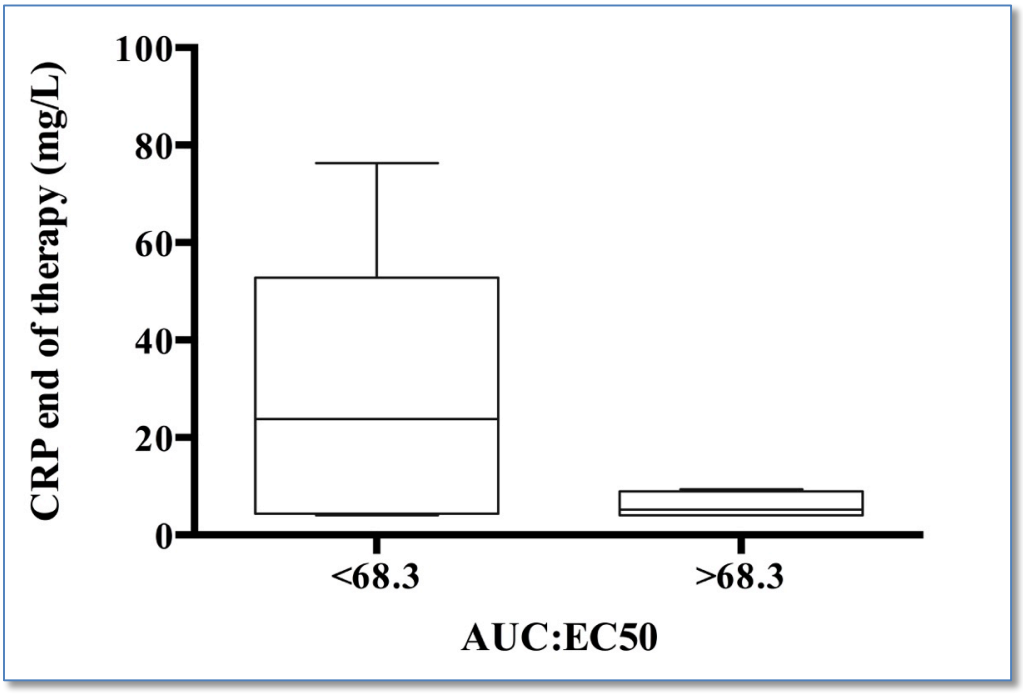


Figure 6.6- Teicoplanin AUC:EC50 box-plot.

The plot suggests that patients attaining >68.3 drug exposures (~>AUC 389.3 mg*h/L) had a more consistently lower CRP at the end of therapy (Mean 18.18 vs 5.7 mg/L), p value=0.002 (two sample t-test).

6.5- DISCUSSION

Teicoplanin is used for the treatment of serious staphylococcal infections (Kacet et al. 1993; Yalaz et al. 2004; Fanos et al. 1997). Currently, teicoplanin is not licensed in the EU for treatment of neonates or infants < 2 months of age because of insufficient evidence data. This PK/PD study provides a rationale to address the appropriate teicoplanin regimen and extent of variability in both drug exposure and response. Furthermore, the study provides the necessary tools to take the next critical steps to provide truly individualised antimicrobial therapy for neonates receiving teicoplanin.

The extent of inter-patient PK variability in this neonatal population was high (figure 6.1a). Of the multiple covariates that were studied, only weight accounted for any portion of the observed PK variability. Incorporation of weight into structural PK models resulted in better fits and statistically more likely solutions. Of note, we could have equally reasonably related weight to clearance using linear or power scaling terms, despite the convention for using a scaling exponent of 0.75 (Hope et al. 2007; Wade et al. 2008). We could not demonstrate any relationship between teicoplanin clearance and PMA, estimated glomerular filtration rate (eGFR) or serum creatinine. This is somewhat surprising because teicoplanin is almost completely renally (98%) cleared by glomerular filtration (A. P. Wilson 2000). The absence of any relationship probably reflects the small sample size as well as the relatively poor estimates of eGFR in neonates using current nomograms. This finding does call into question whether teicoplanin dosing should be adjusted on the basis of eGFR and further studies are required to specifically address this question. Other factors associated with disease, such as variability in regional blood flow, organ perfusion, changes in acid-base balance or cardiac output, might have potentially influenced the drug´s disposition characteristics in our patient population, however, this also requires further and targeted study. To our

knowledge, the pathophysiological mechanisms that describe the pharmacokinetic impact of these covariates and how they relate mathematically with the PK parameters in order to be included in the structural models and differential equations remain poorly defined. Using a non-parametric modelling methodology, we took a pragmatic approach by investigating the clinical parameters known to have a significant impact on our patient population PK variability (weight as an estimate of size, age and serum creatinine/eGFR).

Monte Carlo simulations suggest that the median AUCs at steady state in neonates receiving 16 mg/kg as a loading dose, followed by 8 mg/kg q24 h are comparable to adults receiving 400 mg/day. However, there is much larger PK variability in the AUCs of neonates (Figure 6.4). While the matching of measures of central tendency is straightforward, the best way to match two completely different AUC distributions is less clear. The high variability makes identification of a fixed weight-based regimen challenging because of the unacceptably high proportion of neonates with both low and high drug exposure. Any attempt to address this problem results in an unsatisfactory trade between effect and toxicity and eventual acknowledgment that TDM is required to optimise dosing and drug exposure.

While TDM is the only current way teicoplanin dosing can be optimised, there are a number of significant challenges to this process: first and most obviously obtaining repeated blood draws in premature neonates is never trivial; second, there is persistent uncertainty about drug exposure targets for TDM. A trough concentration of 15 mg/L (measured by FPIA) is proposed in the summary of product characteristics (SPC) by day 3 to 5 of therapy for both adults and children, but recently increased to 20 mg/L and 30-40 mg/L for the treatment of deep-seated infections and infective endocarditis, respectively (The Electronic Medicines Compendium 2014). Moreover,

concentrations are not recommended to exceed 60 mg/L, despite little evidence for any relationship between serum concentrations and toxicity in neonates (Yamada et al. 2014). Such recommendations are based on scant clinical evidence in adult patients and with only a rudimentary understanding of the pharmacodynamics of teicoplanin (Matthews et al. 2007; Sato et al. 2006; Ueda et al. 2012). The use of Bayesian feedback tools for dosage individualisation, which requires the availability of robust population PK models and optimally sampled concentrations, may enable the attainment of desired AUC targets (and surrogate trough concentrations) for any individual patient (Neely et al. 2014).

This study is too small to resolve clinical exposure-response relationships. Inadequate power was further compounded by a Gram-positive pathogen being isolated in only 6/18 (33.3%) of patients. Hence, there was no opportunity to examine the relationship between the magnitude of any traditional pharmacodynamic indices (e.g. AUC:MIC) and outcome. Even in larger datasets, the problem of culture negativity is frequently present. In this situation, most investigators use a population value (e.g. MIC_{90}) to calculate drug exposure for an individual patient. Assuming such patients are infected with the most resistant pathogen is conservative, but necessarily biased. The use of CRP and a novel pharmacodynamic index (the AUC:EC50) circumvents some of these issues. The rationale behind this quotient is that EC50 is an *in vivo* measure of drug potency and the AUC:EC50 is a measure of the exposure of drug relative to the potency of its effect. A major advantage of this approach is that it allows for drug exposure targets that are more individualised for a specific patient. The EC50 (and therefore AUC:EC50) is influenced by both the patient and characteristics of the infecting organism. The EC50 captures the impact of multiple variables on exposure-response relationships (e.g. *in vitro* resistance, high bacterial load, a persistent infective

focus, biofilms and immune response). In contrast, when the measure of potency is the MIC alone, as for AUC:MIC, it is only the organism's characteristics that are considered, and all the other factors implicit in EC50 are ignored. The Bayesian posterior EC50 estimates ranged widely (0.6-18.7 mg/L), which again reflects highly variable pharmacodynamics and *in vivo* potency. In this study, the AUC:EC50 predicted the terminal CRP levels after 5 days of therapy (figure 6.5c) for a majority of patients.

It is important to highlight that rate of change of CRP in the model equation assumes that all the CRP reduction occurs as an effect of the drug, which is not a biological absolute truth as the immune system and other effectors extraneous to the drug influence on this reduction. Despite the model fitted the data well, a clear understanding of its limitation to describe the real clinical setting and interpret the results is required. An association of the AUC:EC50 index with additional clinical outcomes (e.g. improvement of clinical signs, microbiological cure, no need of associated/change of therapy, etc) is deemed necessary for the appropriate evaluation of its clinical validity.

The use of CRP as a biomarker deserves some comment. CRP is widely used in clinical practice to guide anti-infective therapy, but much of that process is informal and intuitive (Ehl et al. 1997; Bomela et al. 2000; Pourcyrous et al. 1993). In this study, we explicitly link teicoplanin serum concentrations and changes in circulating CRP (only referred to drug effect). The measurement of CRP in an individual patient provides a real-time estimate of the response to drug. There are clearly some advantages to such an approach: CRP is quantitative, widely available, well validated, and readily accepted by clinicians. It is the most extensively studied biomarker in neonatal sepsis. In addition, a recent systematic review has showed higher specificity and predictive values at symptom onset and after 24-48 hours than procalcitonin (PCT) in neonatal bacterial

sepsis (Hedegaard et al. 2015). PCT has been investigated mainly in early onset sepsis and with different cut-off values depending on time after birth. Its value in neonates is limited by a marked physiological increase after birth (Turner et al. 2006). The ability to link drug concentrations with a biomarker provides the prospect for truly individualised therapy where the dosing of drug is designed to manage a biomarker rather than a serum drug concentration. However, there are some obvious limitations. CRP is a nonspecific marker of infection and inflammation, and adjusting a dose solely on the basis of climbing CRP may be dangerous if the CRP elevation is a result of Gram-negative bacteraemia as was the case in patients 1 and 7, or the result of a severe non-infectious inflammation as appears likely for patient 16 (Figure 6.3). Thus, to guide teicoplanin dosing, there needs to be confidence that the CRP elevation is a result of a teicoplanin-susceptible pathogen. In our study, we had microbiological evidence of a teicoplanin-susceptible organism in a third of patients. However, there was a high clinical suspicion on this being the case for the remaining patients (clinical, laboratory markers of infection and specific risk factors such as a central line inserted). In our setting, teicoplanin and ciprofloxacin constitute the empirical treatment in the context of central-line associated bloodstream infection. All patients received ciprofloxacin or gentamicin until a blood culture result became available. The other antimicrobial could potentially had an impact on CRP decline in the case of a Gram negative causing microorganism. Nevertheless, CoNS was the most commonly isolated microorganism and teicoplanin was only discontinued in two patients with Gram-negative infection. Interestingly, a recent study has also demonstrated that serial CRP measurements can predict whether an organism is sensitive to the empirical antimicrobial therapy in the first 48 hours of treatment of neonatal sepsis (Patil et al. 2016). These findings now need prospective evaluation.

Despite the potential limitations, this study extends the standard pharmacometric approach whereby the population PK is described, Monte Carlo simulations are performed and *post hoc* analyses such as the probability of target attainment analyses are performed, often using PD targets of questionable clinical significance. While the current approach has limitations because of the non-specificity of the biomarker and the limited biological validity of the model equation describing the rate of change of CRP, the analyses begin to refocus therapeutic arguments on the individual patient, using real data to deliver a regimen that is both safe and effective for the clinical problem in hand.

Chapter 7

Tools for the Individualised Therapy of Teicoplanin for Neonates and Children

3.1 ABSTRACT

Objectives: The aim of the study was to develop a population PK model for teicoplanin across childhood age ranges to be used as Bayesian prior information in the constructed software for individualised therapy.

Methods: We developed a non-parametric population model fitted to PK data from prospectively recruited neonates, infants and older children. We then implemented it in the BestDose multiple-model Bayesian adaptive control algorithm to show its clinical utility. It was used to predict the required dosages to achieve teicoplanin optimal pre-dose targets (15 mg/L) from day 3 of therapy. We performed individual simulations in an infant and a child from the original population, who provided early first dosing interval concentration time-data.

Results: An allometric model that linked weight to clearance and volume of distribution (Ke and V) and incorporating renal function as a power function of eGFR (PNA/creatinine for infants < 3 months), best described the data. The median population PK parameters were as follows: $Ke = 0.03*(wt/70)^{-0.25} * Renal\ (h^{-1})$; $V=19.5*(wt/70)$ (L), being $Renal = eGFR^{0.07}$ (ml/min/1.73m^2) or PNA/creatinine (μmol/L). Increased teicoplanin dosages (i.e range 1.2-2.2 times higher) and alternative administration techniques (extended infusions and/or fractionated multiple dosing) were required in order to achieve the targets safely by day 3 in simulated cases.

Conclusions: The software was able to predict individual measured concentrations and the required dosages and administration techniques to achieve the desired target concentrations early in therapy. Prospective evaluation is now needed in order to ensure that this teicoplanin individualised therapy approach is applicable in the clinical setting.

7.2- INTRODUCTION

The pharmacokinetics (PK) of teicoplanin are highly variable in children and neonates (Ramos-Martin et al. 2014; Ramos-Martín et al. 2016). Weight-based dosing is advocated (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015a). Weight affects estimates of clearance and has been incorporated into structural PK models using linear and allometric scaling functions (Pea et al. 2004; Ramos-Martín et al. 2016; Dufort et al. 1996; Sánchez et al. 1999; Zhao et al. 2015a). Different levels of renal function (quantified in terms of serum creatinine, eGFR) also explain a portion of PK variability in adults and children (Zhao et al. 2015a; Yamada et al. 2012a). However, most of the inter-patient variability in PK remains unexplained (Zhao et al. 2015a; Byrne et al. 2015). Consequently, nomograms based on simple covariates cannot be used to adjust dosages to achieve therapeutic targets that are deemed safe and maximally effective.

The area under the concentration-time curve (AUC) to minimum inhibitory concentration (MIC) is the pharmacodynamic index that best links teicoplanin drug exposure with the observed effect against methicillin-resistant *Staphylococcus aureus* (MRSA) (V. Ramos-Martín, A. Johnson, L. McEntee, N. Farrington, K. Padmore, P. Cojutti, F. Pea, M. Neely, W. Hope, submitted for publication). Nevertheless, the pre-dose concentration (Cmin (mg/L)) is the most widely used measure of drug exposure to guide TDM (Tobin et al. 2010). Cmin ≥ 15 mg/L by days 3-5 of therapy are recommended for most indications (The Electronic Medicines Compendium 2014). These targets have recently increased to 20 and 30-40 mg/L in the Summary of Product Characteristics (SPC) for the treatment of deep-seated infections (bone and joint infections) and infective endocarditis, respectively (The Electronic Medicines

Compendium 2014). Currently recommended targets are based on small retrospective studies in adults that have explored the relationship between teicoplanin trough exposure and clinical outcomes (Ueda et al. 2012; Harding et al. 2000). More recently, daily AUCs >750-800 mg*h/L by day three of therapy have been linked to microbiological cure of adults with MRSA infection (Kanazawa et al. 2011; Hagihara et al. 2012).

In this chapter, we report the development of a non-parametric population PK model of teicoplanin in hospitalised neonates, infants and older children (up to 16 years old). We then describe the application of this model for the construction of software that provides decision support for dose individualisation of teicoplanin. Such an approach enables the achievement of desired drug exposure targets in an optimally precise manner and at anytime during the therapeutic course. This approach constitutes a further extension of our broad goal of developing the tools and knowledge to deliver optimised antimicrobial therapy in neonates and children.

7.3- PATIENTS AND METHODS

7.3.1- Pharmacokinetic study of teicoplanin in children and neonates

Pharmacokinetic data from 57 children (39 children aged 1 month-16 years old and 18 neonates between 26-44 postmenstrual age-PMA- weeks) was available for model building. Patients were prospectively enrolled from two different hospitals in Liverpool (Alder Hey NHS Children´s Foundation Trust and Liverpool Women´s Hospital) over a 20 month-period (April 2013 and January 2015). These PK data have been previously reported in two separate population models (Ramos-Martin et al. 2014; Ramos-Martín et al. 2016). In this study, we combined these datasets to develop a joint population PK model fitted to data from neonates to older children. Such an approach avoids the problem of having multiple pharmacokinetic models for the same drug each with an arbitrarily chosen cut-off value for age or size.

Neonates ≤ 44 weeks PMA (post-menstrual age) received a loading dose of 16 mg/kg followed by 8 mg/kg once daily via a 30 minute- infusion. Children > 1 month of age received three loading dosages of 10 mg/kg every 12 hours, followed by 10 mg/kg once daily via a bolus iv infusion (2-5 min), according to dosages currently recommended by the SPC (The Electronic Medicines Compendium 2014). Plasma samples were collected during the first dosage interval and then at steady state (1, 3, 6, 24 hours post-dose) on days 3-7 of therapy. Neonates < 1000 grams contributed to two samples (alternate time-points) per dosing interval because of constraints on sample volume. The duration of the treatment course was at the discretion of the treating physician. All patients received teicoplanin for proven or suspected methicillin-resistant

staphylococcal (either coagulase negative staphylococci-CoNS- or MRSA) sepsis and/or central-line associated bloodstream infection. Demographic variables included weight, height, age in years, post-menstrual age (PMA) in weeks, postnatal age (PNA) in days, serum creatinine and estimated glomerular filtration rate (eGFR) (Schwartz-Haycock) (Schwartz et al. 1984) were also collected and available for each patient.

Teicoplanin concentrations were measured using a commercially available fluorescence polarization immunoassay (FPIA; Thermo Fisher Scientific, Germany). The limit of quantification (LOQ) was < 3.0 mg/L. The dynamic range was 3-100 mg/L and overall precision (intra and inter-assay) was < 6%. *This method was performed by Kevin Padmore.*

7.3.2- Development of a population PK model

A PK model was fitted to the data using Pmetrics 1.4.2 for R statistical package 3.2.2 that utilises the non-parametric adaptive grid (NPAG) algorithm (Neely et al. 2012). The inverse of the estimated assay variance was used to weight the data. Initially, a standard two-compartment model with time-delimited zero-order intravenous (i.v.) input and first-order elimination from central compartment was developed. The standard model is described by the differential equations 1a and 1b below.

$$\frac{dX(1)}{dt} = R(1) - (Kcp + Ke) \cdot X(1) + [Kpc \cdot X(2)] \quad (1a)$$

$$\frac{dX(2)}{dt} = Kcp \cdot X(1) - Kpc \cdot X(2) \quad (1b)$$

Where X(1) and X(2) represent the amount of teicoplanin (mg) in the central (c) and peripheral (p) compartments, respectively. R(1) is the rate of infusion of drug into

the central compartment (mg/h). The central compartment has volume (Vc) in litres (L), from which there is clearance (CL) in litres per hour (L/h), both represented in equation (1a) as the elimination rate constant Ke (=CL/V) (h^{-1}). The central and peripheral compartments are connected by the first-order rate constants Kcp and Kpc (h^{-1}).

Once the standard model was developed, the potential effect of growth (size) and development/maturity on the PK of teicoplanin was investigated across patients. This was conducted using readily clinically accessible measures, such as weight and age. The Bayesian individual posterior median estimates for clearance and volume of distribution were obtained from the standard model for each patient, and then they were plotted against the covariates of interest to interrogate any possible relationships.

In neonates and children, clearance generally scales with size in a nonlinear manner that is best described using a power function. A scaling exponent of 0.75 is most frequently used (i.e. Clearance is proportional to weight$^{0.75}$) for a number of drugs. In addition, physiological maturation may also affect clearance and this is especially apparent in neonates and young infants (Anderson & Holford 2008; Germovsek et al. 2016). In our model building process, Ke and V were both scaled with weight using linear and allometric scaling terms. Because of the relatively limited sampling strategy (particularly in neonates) we did not explicitly estimate the value of the allometric scaling exponent. As previously reported, weight was referenced to a 70 kg person and related to clearance with a fixed exponent of 0.75 and to volume with an exponent of 1(Anderson & Holford 2008).

The development or maturation effect was studied by using age (years), PMA (weeks) and/or PNA (days). Since a linear relationship was observed between the Bayesian estimates for Ke and V with age (years), a model structure containing this relationship was developed and age (years) was scaled linearly to both clearance and

volume (before and after allometric scaling of Ke with weight). In addition, the effect of age (years) was also studied whilst incorporated into a sigmoidal maturation factor as previously shown (Holford et al. 2013). Age (years) or PNA (days) instead of PMA (weeks) was further studied to account for the entire paediatric age range in the population.

Finally, we explored the relationship between renal function (quantified terms of eGFR and serum creatinine) and estimates of teicoplanin Ke and V in the population. We inspected the relationship of renal function and age (also PMA and PNA) to account for maturational changes in the neonatal period and early in infancy (Rhodin, Anderson, Peters, Coulthard, Wilkins, Cole, Chatelut, Grubb, Veal, Keir & Nick H G Holford 2009). A cut-off age in the maturation of renal function was explored given the plotted eGFR *vs* age relationship seen in the study population. Daily serum creatinine values were collected during teicoplanin therapy to reflect any changes in the renal function during the study. GFR was estimated (eGFR) using the Haycock-Schwartz formula (K*Height/ serum creatinine)(Martini et al. 2003). For this estimation, measures of height or length that were not available from the recruited patients, were obtained from the UK paediatric growth charts as population median height values for the age and gender. In addition, K=0.33 was used for pre-term neonates and K=0.45 for term neonates, as previously reported, to reflect the smaller percentage of muscle mass in pre-term *vs* term infants (Brion et al. 1986; Schwartz et al. 1984).

An inter-occasion variability (IOV) factor was added to the dataset to account for changes in the PK parameters (Ke and V) between the study visits (4 days of therapy)(Karlsson & Sheiner 1993). This might be due to the potential rapid physiological changes over and beyond renal function, which could have an impact on the PK of teicoplanin in this population.

The fit of each exploratory model to the data was assessed using a combination of the following: 1) the log-likelihood value, 2) the Akaike information criterion (AIC) 3) the coefficients of determination (r^2) from the linear regression of the observed-predicted plots before and after the Bayesian step, 4) minimization of bias and imprecisions of the observed-predicted plots; and 5) a visual predictive check (VPC). A model comparison was made using the above-named diagnostics in order to choose the final model.

The final model was fitted to the data using an allometric scaling of weight with Ke (containing both Cl and V) as follows:

$$\frac{dX(1)}{dt} = R(1) - \left(Kcp + \left(Ke0 \cdot \left(\frac{wt}{70}\right)^{-0.25}\right) \cdot Renal\right) \cdot X(1) + [Kpc \cdot X(2)] \quad (2a)$$

$$\frac{dX(2)}{dt} = Kcp \cdot X(1) - Kpc \cdot X(2) \quad (2b)$$

Ke=(Ke0*(wt/70)$^{(-0.25)}$)*Renal (The exponent 0.75 corresponds to (- 0.25) because instead of (CL/70)$^{0.75}$, we have used Ke, which is CL/V); V=(V0*(wt/70));

Renal=eGFR^{pw} or PNA (days)/creatinine if Age< 0.25 years old

Ke0=Ke01 and V0=V01, unless IOV=2, then Ke0=Ke02 and V0=V02, where IOV=1 for the first PK sampling visit and IOV=2 for the second visit.

Where, Ke (Cl/V) is the rate constant of elimination (h^{-1}) including Cl, clearance (L/h) and V, the volume of distribution in the central compartment (L); wt is the patient´s weight (kg); IOV= inter-occasion variability, IOV= 1 was for concentrations <96 hours and IOV=2, for information collected at ≥ 96 hours. The renal function was best described as the estimated glomerular filtration rate (eGFR) (ml/min/1.73 m^2) to a

constant power function (pw), or as the PNA, post-natal age (days) divided by the serum creatinine (μmol/L) if the age was < 0.25 years old (3 months old).

7.3.3- Building the teicoplanin dose optimisation software

We incorporated the final population PK model into a teicoplanin multiple-model Bayesian adaptive dosing controller (the software "cartridge"). The controller is based on the concepts and software (BestDose) developed by the University of Southern California Laboratory for Applied Pharmacokinetics and Bioinformatics (LAPKB) (http://www.lapk.org) (Hope et al. 2013; Neely et al. 2015). The teicoplanin cartridge included the structural final model equations relating input (dosing information) to output (plasma concentrations) and the discrete joint probability distribution of the values of the equation variables (PK parameters) in the population, consisting of a discrete number of support points and their associated probability (the Bayesian prior). The cartridge was implemented in BestDose version 0.2.4 for R, which used the cartridge and each patient´s weight, age, PNA, serum creatinine, eGFR and teicoplanin dosing- concentration data to find the least biased and most precise dosage regimen relative to a target concentration, as previously described (Hope et al. 2013).

7.3.4- Simulations to demonstrate the utility of the dose optimisation software

To show the potential value of the dose optimisation software as a clinical tool, the software was used to predict the required dosage by day 2 of therapy to achieve a pre-determined teicoplanin concentration (15 mg/L) from day 3 in two representative subjects selected from the study population chosen based on age: 1) a critically ill infant

(5 months old-0.46 years old-, 6.3 kg, eGFR 63.84 ml/min/1.73m^2) and 2) a critically ill older child (5.78 years old, 16.3 kg, eGFR 108.41 ml/min/1.73 m^2). We used the past real concentration-time and dosing data from these two patients during the first dosing interval plus a 48h trough (n=4 observations) to predict the optimised dose and infusion time to achieve the desired target concentration safely (i.e avoiding peaks > 60 mg/L, regarded as potentially toxic levels (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015a)). A "past" data-file contained the observed concentrations for each patient. A "future" data-file contained the required timings of future dosages and target, an initial guess of the likely future dose(s) that would be required, as well as the infusion time was prepared. The same patients were also investigated with different simulated age-related average eGFR: 77 and 127 ml/min/1.73m^2, respectively) to evaluate the impact of renal function in the patient´s PK profile (Heilbron, M. A. Holliday, et al. 1991).

The dose optimisation software was tested by comparing the estimated predicted PK profile plot against the observations, as well as by the linear regression of the observed versus predicted concentrations for each individual patient. From the predicted concentrations based on the median individual Bayesian posterior parameter distribution, we calculated the bias, which is equal to the mean weighted predicted error ($\sum$ wpe/N), with wpe = /predicted concentration – the actual concentration)/SD for each prediction/observation, and the % bias. We also computed the imprecision, which is the mean bias adjusted weighted squared error ($\sum$ wspe/N-mwpe^2), and its respective percentage for each patient and each experimental run. The weighted mean individual PK parameter values and an average 24h AUC estimated by the trapezoidal approximation to hourly predictions for each subject were also computed by the software.

7.4- RESULTS

7.4.1- Demographics

The demographics and clinical characteristics of the 57 patients used in the population PK model are summarised in **table 7.1.** The majority of patients (n=23, 40.35%) were recruited from the ICU, in most cases after cardiac surgery. Other subjects included oncologic patients with febrile neutropenia (n=17, 29.8%), general medical (n=8, 28.1%) and cardiac medical conditions (n=1, 1.75%).

A total of 394 PK samples were available for analysis with each patient contributing a mean of 5.3 and 7.6 observations in the neonates and older children, respectively.

Demographic or clinic characteristic	**Median (min-max) [IQR range]**		
	Children >1 month	**Neonates**	**Total**
n. of patients	39/39	18/18	57
Mean observations per patient	7.6	5.3	6.9
Gender (male:female)	21:18	12:6	33:24
Wt (kg)	14.8 (3-62.2) [16.7]	2.04 (0.69-5.08) [2.07]	7.5 (0.69-62.2) [16.4]
Height (cm)	97.9 (45-170)* [49.25]	48 (36-52)** [11.5]	72 (36-170) *** [65]
Age (years)	3.3 (0.12-15.8) [6.13]	0.05 (0.01-0.19) [0.04]	0.88 (0.01-15.82) [4.9]
PMA (weeks)	NA	37(26-44) [10.25]	NA
PNA (days)	1204.5 (43.8-5774.3) [2237.5]	17(4-69) [15.5]	321.2 (4-5774.3) [2078]
eGFR (ml/min/1.73m^2)	78.94 (6.43-160.3) [50]	42.8 (5.4-95.2) [25.5]	62.06 (5.4-160.3) [49.03]
Serum Creatinine (μmol/L)	41 (27-308) [14]	44.5 (21-265) [21.75]	41 (21-308) [15.5]

Table 7.1- Demographics and clinical characteristics of patients included in the population PK model. *n=30; **=UK Pc50 length for gender and age; ***n=30 from the patients, the remaining unavailable data for height or length was obtained from the UK paediatric growth charts as the Pc50 height (median value) corresponding to the gender and age. NA=not-applicable.

7.4.2- Population PK of Teicoplanin in Neonates and Children

The population PK allometric model (Ke and V with weight) better accounted for the observed data than the linearly scaled model and was chosen to build the dose controller. This model included a descriptor of renal function that was incorporated as a power function term for eGFR ($eGFR^{pw}$) or as PNA/creatinine. **Figure 7.1** shows the relationships obtained in the exploratory process of building the structural model. The final population PK model explained 28 % of the PK variability in the population (16% more with the incorporation of the covariates). PNA/creatinine was used for neonates and infants < 3months of age (0.25 years old) as a better descriptor of renal function given the two different patterns observed around this age in the eGFR *vs* age plot (**Figure 7.1 d.2 and e.1**). A model comparison and diagnostics between the standard model without covariates, an exploratory model (adding a linear relationship between age and Ke), and the final model is shown in **table 7.2.** For the final model, the linear regression of observed versus Bayesian-predicted values had a coefficient of determination of $r^2 = 0.92$ with measures of bias and precision of -0.15 mg/L and 0.9 mg^2/L^2, respectively. The population and posterior individual observed versus predicted plots of the final model are shown in **Figure 7.2.** A visual predicted check (VPC) based on 1000 model-predicted simulated profiles of a subject with homogeneous dosing history and sampling schedule (with limit ranges to clinical covariates as per the original population) is shown in **Figure 7.3**. The mean, median and standard deviation of the final model population PK parameter estimates are shown in **table 7.3.**

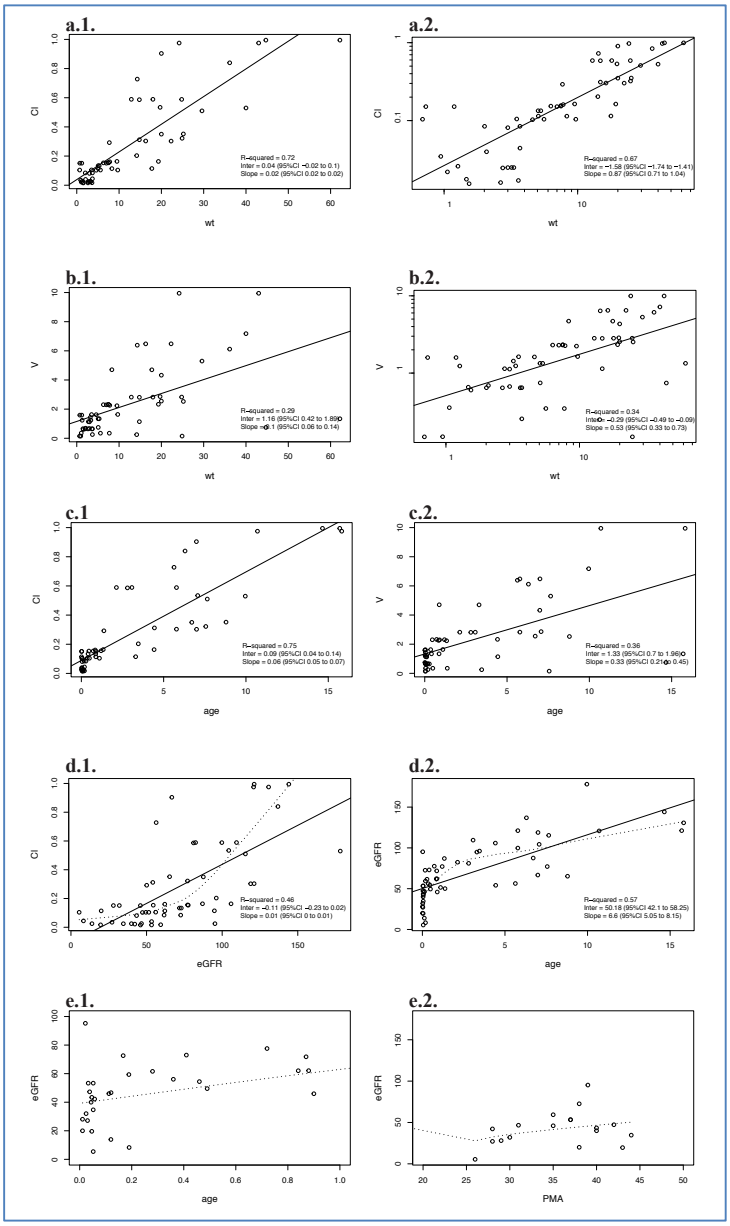


Figure 7.1- Model building exploration of relationships between individual Bayesian posterior estimates for clearance (Cl) and volume (V) from the standard model and the clinical covariates.

a) linear and logarithmic relationships between Cl and weight (wt); b) linear relationship between V and wt; c) Linear relationship between Cl, V and age; d) Cl and eGFR, eGFR and age (years); e) eGFR and age (up to 1 year old) establishing the age cut-off value for the different pattern of the power function relationship. The scatterplot between eGFR and PMA (weeks) is also shown (e.2). The continuous line shows the linear regression line and the dashed line shows the LOWESS (locally weighted scatterplot smoothing) or local regression line, which highlights a Cl-eGFR relationship compatible with a power function, where GFR is the independent variable raised to a constant (pw) in d.1.

Model	Standard (without covariates)	Final model (allometric without age)
N. of variables	4	7
Log-likelihood value	2523	2360
AIC	2533	2376
Pop/post Bias	3.8/-0.02	1.1/-0.15
Pop/post Imprecision	72.2/1.5	12.3/0.9
Pop r^2	0.12	0.9
Post r^2	0.8	0.92

Table 7.2- Model comparison and model diagnostics with and without covariates.

AIC: Akaike information criterion; Pop/post Bias: population and posterior mean weighted error respectively; Pop/post Imprecision: population and posterior mean bias-adjusted weighted squared error; Pop/Post r^2 = coefficient of determination for the linear regression of the observed *vs* predicted plots for the population and the posterior fits, respectively.

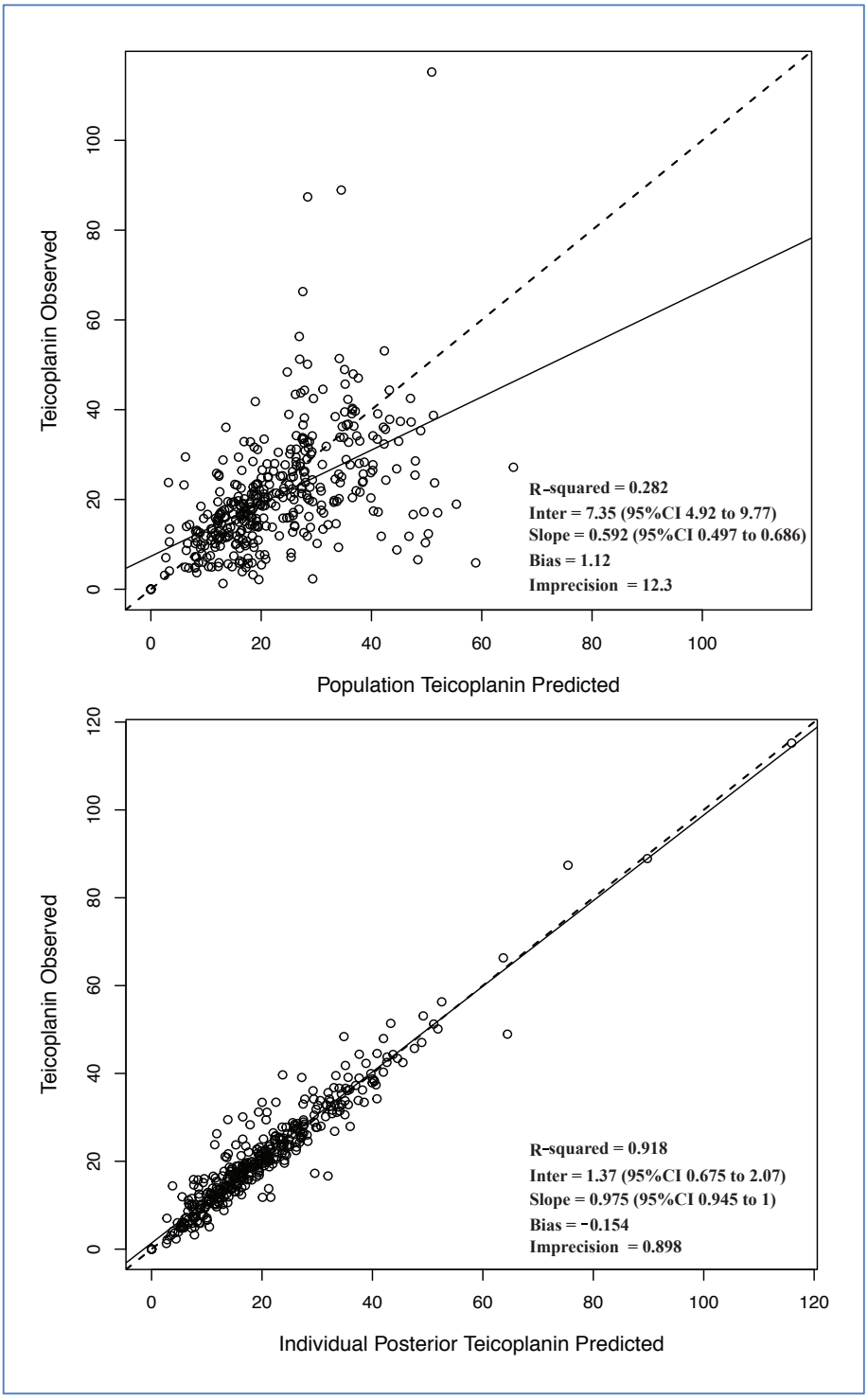


Figure 7.2- Observed-predicted concentrations scatter plots for the final Population PK model.

Before (population) (top) and after (individual posteriors) the Bayesian step (bottom).

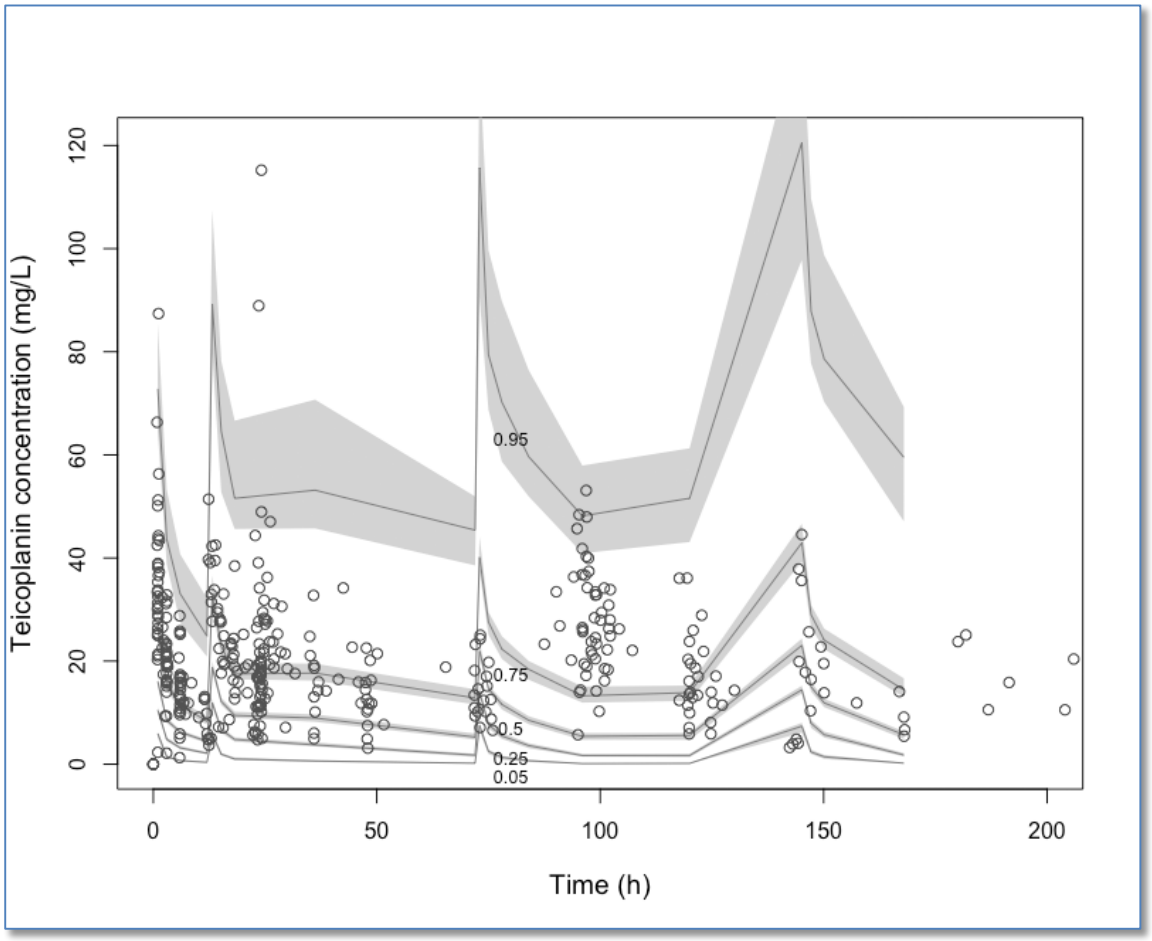


Figure 7.3- Visual Predictive Check for the population PK model.

Continuous lines represent the model predicted-simulated PK profiles as (0.05,0.25,0.5,0.75,0.95) quantiles, grey areas are the 95% CI and empty circles the overlaid observations.

Parameter	Ke01 (h^{-1})	Ke02 (h^{-1})	V01 (L)	V02 (L)	Kcp (h^{-1})	Kpc (h^{-1})	pw
Mean	0.038	0.036	22.636	22.472	0.490	0.214	0.125
Median	0.03	0.025	19.52	22.7	0.23	0.12	0.07
SD	0.04	0.03	14.23	7.95	0.48	0.27	0.15

Ke= Rate constant of elimination= $Ke0*(wt/70)^{-0.25}$ *Renal; $Renal=eGFR^{pw}$ or PNA/creatinine if < 0.25 years old; V=Volume of the central compartment=V0*(wt/70); Kcp and Kpc are the first-order intercompartmental rate constants; pw=power function. The epithet 01 and 02 denote the occasion. Ke0=Ke01 and V0=V01 for time < 96 hours; otherwise, Ke0=Ke02 and V0=V02.

Table 7.3- Population PK parameter estimates from the final model.

7.4.3- Performance and simulations to demonstrate the clinical utility of the teicoplanin dose optimisation software

The dose optimisation software predicted the PK profile of the individual patients and achieved a target with minimal bias and imprecision. The **table 7.4** shows the bias, % bias, imprecision, % imprecision and the coefficient of determination (r^2) of the linear regression of the observed *vs* predicted measured concentrations. Individual weighted mean PK parameter values were obtained. The median (range) average daily $AUC_{0\text{-}24}$ for each patient is shown.

Figures 7.4 and 7.5 show representative plots from the dose controller for the infant and the older child, respectively. They represent the software predicted PK profiles with their respective measured concentrations (red circles) and the target concentration (green squares). In the case of the infant, all required optimised doses by day 2 of therapy and a day 3 pre-dose target of 15 mg/L were 1.8 to 2.2 times higher than currently recommended maintenance dose (10 mg/kg) for 12-h extended infusions and a 24-hourly bolus administration, respectively (**Fig. 7.4a and 7.4b**). The regimen with daily 24-hour infusions required similar and subsequent slightly decreased dosages (**Fig. 7.4c**). Increased dosages and differing infusion times did not reach peak concentrations >60 mg/L, except for the first case (bolus administration by day 2 of therapy). In the case of the 5-year old child, a higher bolus administration of 18.4 mg/kg (as opposed to 10 mg/kg maintenance dose) was required for the day 3 pre-dose 15 mg/L target, although producing peaks > 60 mg/L (**Fig. 7.5a**). In this patient, extended 12 and 24-h infusions and even a multiple fractionated 12-hourly dose would have been safer alternatives (**Fig. 7.5b, c, d**). Increased predicted and optimal dosages were from 13.9 mg/kg (1.4 times higher) to 3.7, 2.6 and 2.2 mg/kg for the multiple fractionated doses and pre-dose targets of 15 mg/L. There was not a significant impact, when the

simulated patients had an average age-related eGFR, higher than actual estimates. In these particular cases, they both required slightly increased dosages (≤2%).

Subject	**1_Infant**	**2_Child**
r^2	1	0.97
Bias	-0.42	0.4
% Bias	-2.54	8.46
Imprecision	0.3	1.64
% Imprecision	0.05	1.54
Median (range) of average 24h AUC (mg*h/L) along the treatment course	493.8 (355.8-574)	368.1 (318.9-388.4)

r^2= coefficient of determination of the linear regression of the predicted *vs* observed concentrations; Bias=mean weighted predicted-observed error; % Bias= 100* [mean weighted predicted-observed error/observed]; Imprecision= bias-adjusted mean squared error; %Imprecision= 100*[bias-adjusted mean squared error/observed].

Table 7.4- Summary of the individual prediction diagnostics from the dosing optimisation software for the two patients (using past-real data to obtain a target of 15 mg/L from day 3 of therapy).

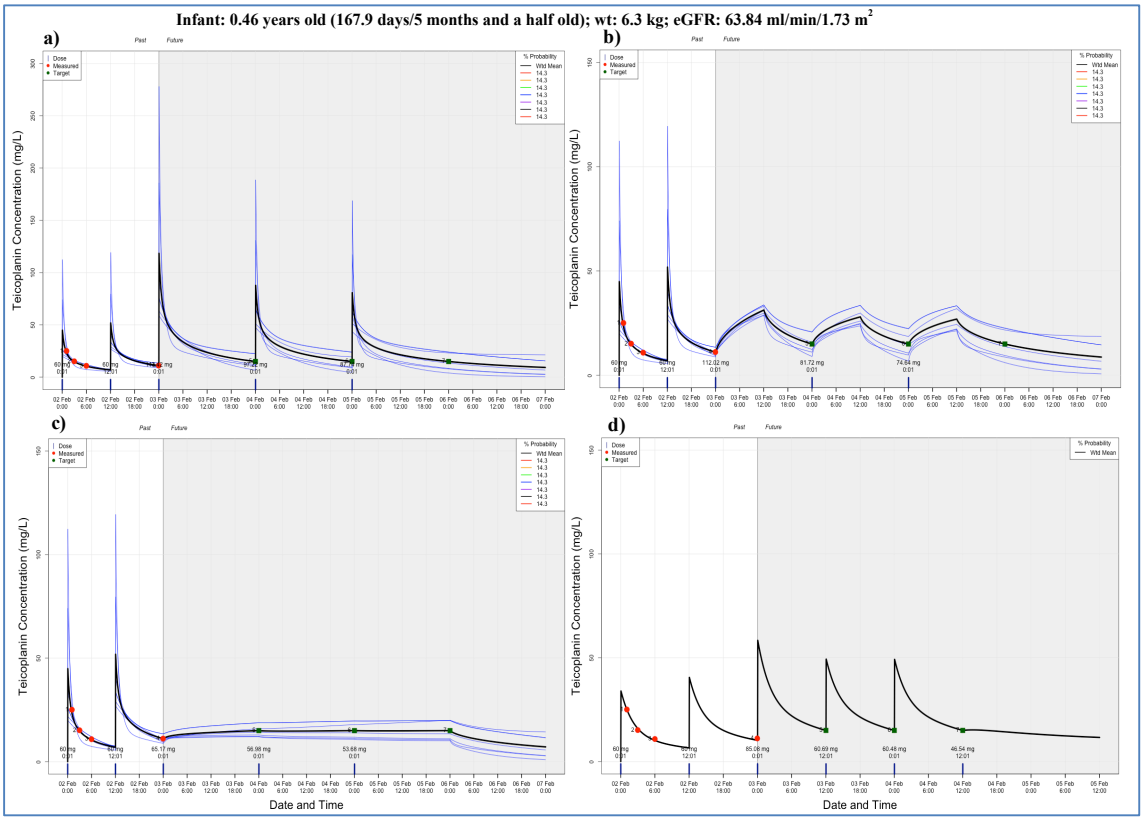


Figure 7.4- Representative plots from an infant patient receiving teicoplanin.

Pre-dose target of 15 mg/L from day 3 of therapy following a) bolus administration; b) 12h infusion; c) 24h infusion and d) multiple 12-hourly dosing.

[a) Bolus administration: Dose required by day 2: 143.4 mg (22.3 mg/kg); day 3: 97.22 mg (15.4 mg/kg); day 4: 87.74 (13.9 mg/kg); b) 12h infusion: Dose required by day 2: 112.02 mg (17.8 mg/kg); day 3: 81.72 mg (12.97 mg/kg); day 4: 74.64 mg (11.8 mg/kg); c) 24 h infusion: Dose required by day 2: 65.17 mg (10.3 mg/kg); day 3: 56.98 mg (9.04 mg/kg); day 4: 53.68 mg (8.5 mg/kg); d) Multiple dosing every 12 h: Doses required by day 2: 85.08 mg (13.5 mg/kg), and every 12 h thereafter 60.69 mg (9.6 mg/kg) and 60.48 mg (9.6 mg/kg) and 46.54 mg (7.4 mg/kg)]**.**

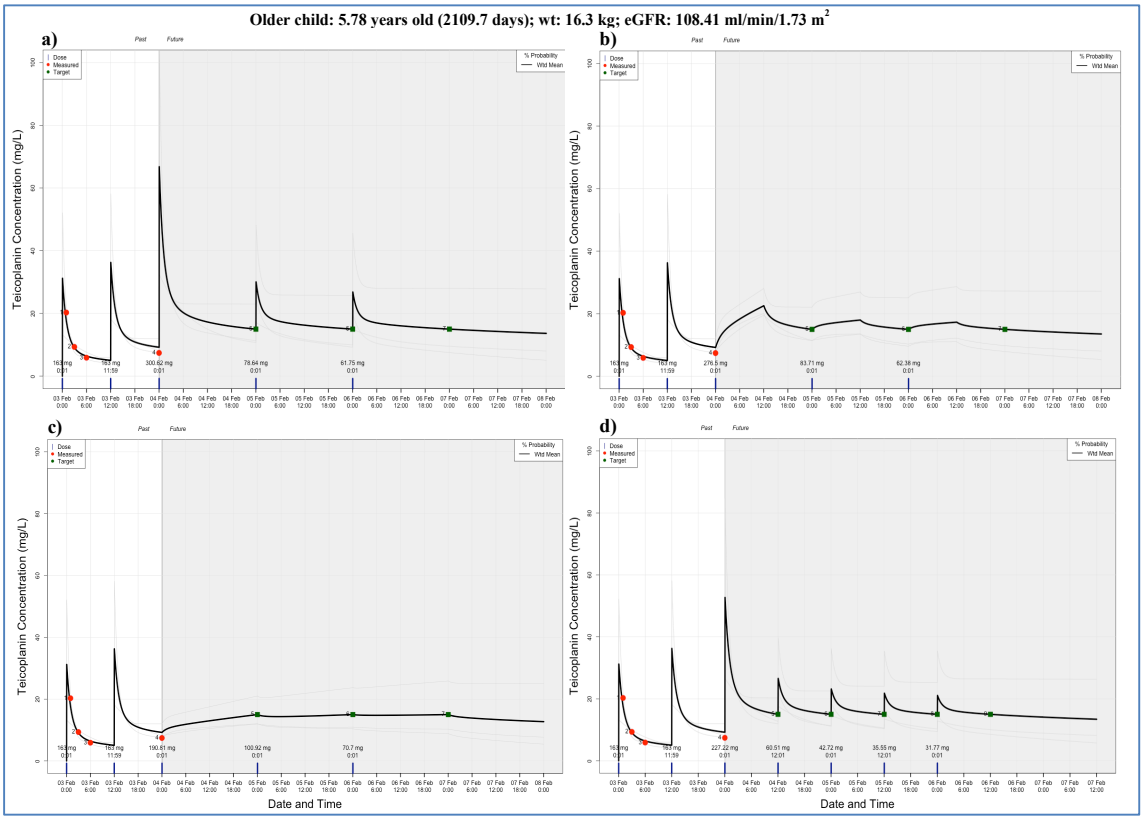


Figure 7.5- Representative plots from an older child patient receiving teicoplanin.

Pre-dose target of 15 mg/L from day 3 of therapy following a) bolus administration; b) 12h infusion; c) 24h infusion and d) multiple 12-hourly dosing.

[a) Bolus administration: Dose required by day 2: 300.62 mg (18.4 mg/kg); day 3: 78.64 mg (4.8 mg/kg); day 4: 61.75 mg (3.8 mg/kg); b) 12h infusion: Dose required by day 2: 276.5 mg (17 mg/kg); day 3: 83.71 mg (5.1 mg/kg) and day 4: 62.38 mg (3.8 mg/kg); c) 24h infusion: Dose required by day 2: 190.81 (11.7 mg/kg); day 3: 100.92 mg (6.2 mg/kg) and day 4: 70.7 mg (4.3 mg/kg); d) Multiple 12-h dose administration: Doses required by day 2: 227.22 mg (13.9 mg/kg); and 12-hourly, thereafter, 36 h: 60.51 mg (3.7 mg/kg); 48 h: 42.72 mg (2.6 mg/kg); 60 h: 35.55 mg (2.2 mg/kg); 72 h: 31.77 mg (1.9 mg/kg)].

7.5- DISCUSSION

An improved understanding of the PK/PD of teicoplanin is fundamental to the optimal use of this agent (G.L. Drusano 2004). In this study, we developed a population PK model in neonates, infants and children to identify PK variability and its explanatory clinical covariates within a heterogeneous cohort. Importantly, with the available patient covariates, the model can only account for 28% of the observed variability in teicoplanin concentrations in this population (Figure 7.2, top), which is consistent with other population PK studies in children and adults (Zhao et al. 2015a; Byrne et al. 2015). In contrast, combining the model with individual patient measurements permits accurate prediction of concentrations (Figure 7.2, bottom), a strong argument for the use of TDM to control dosing and avoid sub-optimal antimicrobial exposure that will result from the wide distribution of drug exposures (Ramos-Martin et al. 2014; Ramos-Martín et al. 2016). Pharmacometric approaches to TDM (i.e Bayesian calculations) are best suited to aid in the optimised and truly individualised use of antimicrobials in general, and of teicoplanin in particular (Macdonald et al. 2008).

For other antimicrobial agents, such as vancomycin, aminoglycosides and voriconazole, the rationale for TDM is compelling because there are generally accepted exposure-efficacy and exposure-toxicity relationships. For teicoplanin, a relationship between plasma concentration and toxicity has not been established (Tobin et al. 2010; Matthews et al. 2014; Yamada et al. 2017; Yamada et al. 2014), which may reduce the incentive for physicians to routinely monitor the drug. However, exposure control to maximize efficacy should not be neglected. The British National Formulary for Children (BNFC) suggests a therapeutic window of >15 to 60 mg/L for children and adults (BMJ Group, the Royal Pharmaceutical Society of Great Britain 2015a). This window is based on retrospective associations, particularly against MRSA infection

(Harding et al. 2000; Strenger et al. 2013; Ueda et al. 2012; Matthews et al. 2014). Moreover, low drug exposures increase the probability of clinical failure and development of drug resistance (George L. Drusano 2004; Chang et al. 2012).

Teicoplanin dosage regimens that are stratified by weight and/or renal function improve the achievement of target concentrations in adults (Yamada et al. 2012b; Niwa et al. 2010). However, high and unexplained PK variability in children and neonates, make steady-state empirical dose adjustment 3-5 days after starting teicoplanin or changing the dose, a sub-optimal strategy that can result in a high proportion of sub-therapeutic concentrations < 10 mg/L (i.e 55-89%)(Dufort et al. 1996; Sánchez et al. 1999; Strenger et al. 2013). This can result in prolonged and unnecessary patient exposure outside the optimal range. Hence, an optimal way of performing patient-tailored TDM would rapidly achieve therapeutic concentrations with minimal sampling and avoidance of steady-state requirements.

Bayesian tools offer just such a way to achieve target concentrations as rapidly, accurately and precisely as possible (Macdonald et al. 2008). The Bayesian adaptive multiple-model control algorithm was used in this study to: 1) update the general paediatric population teicoplanin´s PK parameter probability distributions for an individual patient, and 2) to use the updated, individual model to match measured concentrations, predict future concentrations, and calculate dosages to achieve target concentrations, all without the need for steady state or even precisely timed trough samples.

In the clinical setting, there are several difficulties that can hamper the optimal application of the feedback dosing control. Intra-individual, inter-occasion variability (IOV) (Karlsson & Sheiner 1993) can cause unexplained PK variability and poor model predictions, particularly in neonates and/or critically ill patients who are unstable

(Holford et al. 2013; Blot et al. 2014). Quantification of rapid pathophysiological changes, such as renal function, and subsequent incorporation as covariates into the PK model may allow better prediction of intra-individual variability in this population. We found little IOV for teicoplanin in this population. A bedside feedback control tool allows updated teicoplanin concentration-time data and patient´s covariate information to be re-entered into the software on an iterative way. The algorithm we used in the BestDose software permits one to escape the assumption that PK parameter values are constant in a patient, making it ideal to capture IOV(Macdonald et al. 2008). This can account for real intra-patient variability during therapy.

Another interesting aspect of our work was the use of post-natal age (PNA) divided by serum creatinine (SCr) as a novel marker of renal function. When modelling and controlling drug behaviour, it is not necessary to describe renal function using traditional equations that estimate GFR, although we did use the Schwartz equation for the infants >3 months of age. The goal in pharmacometrics is to find the best descriptors of drug behaviour, in this case related to renal function and maturation. We feel that PNA/SCr has advantages over any other estimation of renal function, including Schwartz, in that it does not use length (height), which can be notoriously inaccurate in infants. We have previously found PNA/SCr to be a useful predictor for both vancomycin (Yamada et al. 2015) and gentamicin PK (Neely & Floyd 2015) in infants.

In conclusion, we present a tool to rapidly and accurately predict teicoplanin concentrations and calculate doses that optimally achieve desired concentrations in paediatrics. We further validate PNA/SCr as a novel predictor of renal drug elimination in neonates and young infants. A prospective study evaluating the cost-effectiveness of such individualised dosing is now warranted.

Chapter 8

General Discussion and

Final Conclusions

8.1- PROJECT OVERVIEW: A translational pharmacological approach to optimise antimicrobial therapy in children

This thesis provides novel insights into our understanding of the exposure (PK)-response (PD) relationships of two well-established glycopeptide agents (vancomycin and teicoplanin) against the most prevalent causative pathogens of BSI in neonates and children (namely CoNS and MRSA). This constitutes the first critical step for optimising the clinical use of these currently available antimicrobial drugs in children.

This step could be achieved with the use of a translational pharmacological approach that combined: 1) the use of pre-clinical infection models, both *in vitro* and *in vivo* experimental models, 2) the collection of clinical pharmacokinetic and pharmacodynamic information (e.g CRP concentrations) from the target population (neonates and children) and 3) mathematical modelling techniques that allowed a robust analysis of pre-clinical and clinical exposure-response information, the identification and quantification of variability and the bridging of experimental findings to patients (by means of Monte Carlo Simulations). Finally, we applied the developed population clinical PK model across childhood (a summary of prior past information) within a Bayesian adaptive control algorithm (BestDose) to allow the individualised therapy of teicoplanin.

The pre-clinical models of infection permitted defining the most relevant PD index as well as the magnitude of that index that best linked vancomycin and teicoplanin drug exposures with antimicrobial efficacy. This is crucial for setting up the experimental basis of an optimal use of the antimicrobial of study (both in terms of bacterial killing and preventing/suppressing the emergence of resistant subpopulations).

In line with our initial project aims, several key findings were made during this PhD project:

1) Vancomycin displays a concentration-dependent (AUC:MIC) activity against CoNS infection (Chapter 3). This observation was achieved in both *in vitro* and *in vivo* pre-clinical infection models used in the project to mimic a neonatal BSI and a CLABSI (*in vivo*) caused by CoNS. An AUC:MIC ratio was the best pharmacodynamically linked index predictive of efficacy against a total bacterial population and a resistant (mutant) sub-population. The study suggested that less fractionated vancomycin regimens are appropriate for clinical use, and that continuous infusion may be associated with increased risk of emergence of resistance. The *in silico* bridging study to human neonates concluded that pre-term neonates < 29 weeks PMA were under-dosed in comparison with the older age-groups. These findings provided the PK/PD evidence to inform an optimised neonatal dosage regimen to take into a randomized controlled trial.

2) Teicoplanin also displayed a concentration-dependent (AUC:MIC) activity against MRSA infection (Chapter 4). This observation was concordant using two pre-clinical (*in vitro* and *in vivo*) infection systems. A simulation-based PTA analysis in adults and children showed that the PK-PD cut off was much lower than the currently defined EUCAST MRSA clinical breakpoint for teicoplanin (S≤ 2 mg/L)(European Committee on Antimicrobial Susceptibility Testing, 2010). This suggested that current licensed dosage regimens might be unlikely to treat patients successfully (both adults and children) in monotherapy; particularly, those infected with strains with MICs at the higher end of the current susceptibility range. Conclusively, established breakpoints might need to be revised with more supportive, updated and comprehensive pre-clinical and clinical datasets.

3) Clinical population PK studies of teicoplanin allowed the collection and analysis of clinical PK profiles to develop a population PK model in neonates and children (Chapter 5 and 6). In the case of the neonates, CRP information was also incorporated to the model as the linked PD input (Chapter 6). The population PK (and PD when linked to CRP) model revealed a high PK (and PD) variability in the population. An AUC:EC50 ratio was explored to account for a more informative PD target in the neonates, where results from blood cultures and thus, MICs, are rarely available. Weight, as a surrogate of size, age and renal function (eGFR or PNA/creatinine) were the most explanatory covariates of such PK variability (Chapters 5, 6, 7). Nonetheless, a high proportion of the PK variability in neonates, infants and children was unexplained and not accounted for by clinical covariates. This supports the mandatory use of teicoplanin TDM to ensure adequate drug exposures in individual patients.

4) Teicoplanin individualised therapy (Chapter 7). Given the high and unexplained PK (and PD) variability in children, the individualised dosing and TDM of teicoplanin is required to maximise its efficacy, minimise toxicity and improve the clinical outcomes in this population. We showed the clinical utility of a Bayesian adaptive feedback control algorithm (BestDose) to individualise dosing in paediatric patients providing early concentration-time observations. Further and prospective cost-effectiveness evaluation of this tool for dose individualisation is now required.

8.2- THE ROLE OF PK/PD DETERMINATION AND ANALYSIS IN OPTIMISING ANTIMICROBIAL THERAPY

Laboratory-based *in vitro* and animal data are used to define antimicrobial PD, which supports the knowledge and understanding of relevant PK/PD indices descriptive of maximal activity. PK/PD relationships established in pre-clinical studies are predictive of therapeutic responses in humans (Craig 1998). Although these indices may be helpful to inform dose optimisations in different clinical scenarios, more research is warranted to validate the clinical relevance of PK/PD relationships observed *in vitro and in vivo*.

In Vitro PK/PD Studies

A HFIM *in vitro* model was used throughout the pre-clinical chapters of this thesis (Chapters 3 and 4). This model enabled the investigation and quantification of the bacterial killing-drug effect, as well as the effect against the emergence of drug resistance under drug pressure. The beauty of this model relies in the possibility of exposing the infective bacteria to daily changing drug concentrations that are continually adjusted to mimic either a neonatal or a child PK human profile *in vivo*. The main limitation of this *in vitro* model, is a lack of immune system, which provides a caveat in the interpretation and translation of results to patients. Nonetheless, it also means that a drug regimen which is active *in vitro* (or in a neutropenic animal) is more likely to be effective in immunosuppressed patients, such as pre-term neonates, critically ill neonates, young infants and children, as well as febrile neutropenic oncologic children.

A high inoculum size above the strain mutational rate together with a delay of 24 hours to initiate therapy was necessary (i.e reaching inocula sizes ~ 8-9 Log_{10} CFU/mL)

to provide information with respect to the emergence of resistance. These experimental conditions *in vitro* might reflect the worst clinical scenario in a patient, where information about clinically relevant bacterial densities for a BSI in a neonate/child is not known. An *in vivo* model can place a more clinically relevant (i.e sub-lethal) bacterial load in context, however, the correlation with the patient and the need for a probable more conservative drug exposure target needs to be investigated.

The lack of appropriate comparable protein binding in microbiological growth media (MH broth and agar) to human serum constitutes another main limitation of *in vitro* models. Since the physiological conditions (pH, electrolytes, temperature, source of proteins, fatty acids, etc) differ, the quantitative determination of the impact of protein binding *in vitro* and *in vivo* remains challenging. The current diversity of models, methods and results looking at the impact of protein binding on antimicrobial therapy, particularly for highly protein-bound molecules, contribute to the ongoing debate about its relevance and understanding (Zeitlinger et al. 2011). Further and more standardised targeted research in this particular area is warranted. In the meantime, measurement of protein binding in the experimental medium (and in the animal species of study) is desirable in an attempt to mimic physiological conditions as thoroughly as possible.

In vivo PK/PD Studies

Animal infection models provide the opportunity to study drug efficacy while accounting for the host immune response, virulence of the pathogen and antimicrobial susceptibility linked to bacterial load. In this thesis project, the CLABSI rabbit model and the mouse neutropenic thigh infection model were closer mimics of human disease/response whilst also accounting for total (protein-bound) drug exposures. They

both provided complementary information to the HFIM system and validated the *in vitro* findings. Additionally, the CLABSI rabbit model permitted the evaluation of the complexities of a central-line associated infection in a critically ill neonate context, where the line needs to be retained in the patient and staphylococcal biofilm develops.

The assessment of a biomarker of antimicrobial response (circulating CRP) was also assessed in the rabbit infection model. This was interesting from two points of view. The first one, it was a necessary PD readout information for the evaluation of exposure-response relationships. The blood cultures were not informative as they displayed highly variable results. This is in line with what happens in the clinical setting for neonates, where constraints in the blood volume of samples for blood cultures can yield insensitive results (Connell et al. 2007). Second, it enabled the incorporation of a quantitative PD measure, widely used and validated in the clinical setting, into the structural linked PK/PD mathematical models. The use of biomarkers (i.e CRP, procalcitonin) have proved to be essential to guide initiation and discontinuation of antimicrobial therapy (Bomela et al. 2000; Nouér et al. 2011). However, further evaluation is still needed to determine the potential benefits of biomarker modelling in the management of patients, as well as in the individualised dosing and TDM approach (Huurneman et al. 2016). Importantly, biomarker changes might not be highly specific for the treated infection and there needs to be confidence that its dynamics reflect the interaction between drug, pathogen and host response. Existing additional factors (e.g. surgery and tissue damage) affecting the biomarker response need to be considered in a more advanced modelling framework.

Finally, there still exists some uncertainty as if the pre-clinical infection models can reproduce adequately the potentially altered and variable PK and PD, particularly observed in pre-term neonates, low birth weight neonates and critically ill patients. *In*

vivo models allow for assessing multiple endpoints including mortality, quantitative bacterial counts in blood/ tissues and description of inflammatory responses (e.g. CRP, cytokine measurements). However, there still exist important differences between species and different patient-populations and sub-populations (e.g. the host immune response, the protein-binding affinity, the relevant endpoint) that can affect the clinical relevance of animal data.

In many instances, the magnitude of exposure identified for stasis in immunocompromised animals have been similar to the exposure threshold associated with good clinical outcomes in patients (Ambrose et al. 2007). A more in-depth understanding of the appropriate extrapolation of magnitudes of exposures from pre-clinical models into the patients is necessary. PK/PD data derived from patients will provide the opportunity to confirm knowledge gained from pre-clinical PK/PD infection models.

PK/PD Population Mathematical Modelling of Experimental Data and Bridging to Patients

Population PK/PD modelling was conducted to describe, identify and quantify the exposure-effect relationships from both pre-clinical and clinical systems. In the case of modelling the experimental data, the aim was defining the PK/PD relationships and investigating the PD targets predictive of maximal efficacy and suppression of a resistant subpopulation. This was achieved by means of fitting the final model-Bayesian predictions (i.e for the time-course of drug exposures and bacterial density/CRP at the end of therapy) to an Emax inhibitory sigmoid model (Hope & Drusano 2009).

Population PK/PD modelling methods can be statistically classified as either parametric or non-parametric. Each classification can be divided into maximum likelihood (ML) or Bayesian approaches (Tatarinova et al. 2013). Throughout the project, the non-parametric population modelling software Pmetrics was used. This software uses a combination of NPEM (non-parametric expectation maximization) for the first cycle of the model fitting process, with the NPAG (non-parametric adaptive grid) algorithm of Leary and Burke (Neely et al. 2012). This NP approach does not make any assumptions about the underlying distribution of the PK/PD parameter values and use an exact computation of the likelihood (ML). For clinical data, the assumption that parameter estimates are non-parametrically distributed may be appropriate and enables the detection of sub-populations and outliers within the study population (Neely et al. 2012). This could be especially relevant in very heterogeneous populations where a small sub-group of patients (e.g very low birth pre-term neonates) could have quite different clinical characteristics that could influence the drug´s disposition and/or effect (e.g rapidly changing renal function or an immature immune system).

In previous comparative performance studies of parametric and non-parametric population methods in analysing clinical data, non-parametric methods were better than parametric ones at analysing populations having unanticipated non-Gaussian or multimodal parameter distributions. In addition, using exact or accurate computations gave more precise parameter estimation, showed better stochastic convergence properties and proved more statistically consistent (the more subjects studied, the closer the estimated parameter values approached the true ones) as opposed to parametric algorithms that are not based on maximum likelihood but approximated functions, eg. FO, FOCE, FOCEI (first order conditional expectation)(Bustad et al. 2006).

For analysis of *in vitro* and *in vivo* PK/PD models, where "individuals" are more homogeneous and behaviour is highly controlled, parameter estimates may be normally or log-normally distributed making the non-parametric analysis less necessary. There are no published comparisons of non-parametric (e.g Pmetrics) with parametric ML software programmes (e.g NONMEM, Monolix and ADAPT) for the analysis of experimental PK-PD infection model data.

Limitations/weaknesses of the non-parametric modelling approach used in this project

In order to complete a non-parametric analysis Pmetrics requires a fixed error model to weight the observations. In Pmetrics, each observation is weighted by the inverse of the variance (=1/error^2). The error model is derived from the assay variance, which can be easily measured for PK studies but is more difficult to estimate for PD data ($\sqrt{\text{variance}} = SD = C_0 + C_1*[Observation] + C_2*[Observation]^2 + C_3*[Observation]^3$). Additionally, the error model is multiplied by a scalar (γ), which is estimated by Pmetrics and represents the environmental error or process noise not included in the assay variance (Error= SD* γ)(Jelliffe et al. 2000). Inclusion of this scalar, therefore,

assumes that all the error in the data or model structure is proportional to the assay variance, which it may not be. This is in contrast to alternative parametric modelling software options where the error is estimated in the analysis.

In addition, in Pmetrics there is no feature to separate the various sources of variability into their respective components. For example, the inter-individual variability (the diversity among the subjects in the ways they handle the drug) and the intra-individual variability (e.g rapid physiological changes in unstable patients and subsequent changing parameter values during the study period) from the environmental error (i.e the errors in preparing and giving the doses, recording the times at which serum concentrations were obtained, the assay error itself, the model misspecification, etc). On the contrary, parametric methods, such as NONMEM, are able to split the various sources of variability, whilst allowing two levels of random effects (noise): inter-individual and residual variability (Standing 2016).

At the start of the project, a non-parametric or semi-parametric Monte Carlo Simulator was not available in Pmetrics. Thus, for Chapter (3) and (5), the population and individual simulations were performed using ADAPT 5, which assumes that the parameter estimates are normally or log-normally distributed. The impact of using parameter values estimated using a non-parametric method in a parametric simulator is unknown. Subsequently, a general-purpose Monte Carlo simulator within Pmetrics was used. This simulator samples new sets of parameter values from prior parametric or non-parametric distributions from the developed PK/PD model. From each sampled set of parameter values, the simulator calculates observations based on a template of different dosing inputs, output measurement times and relevant covariates.

8.3- CLINICAL PK STUDIES IN CHILDREN AND NEONATES

Collecting concentration-time data of antimicrobials in children and neonates is essential for developing population PK models, which can be progressed into dosing regimens that are appropriate for the intended population and infection syndrome.

For this thesis project, we conducted a clinical PK study of teicoplanin in prospectively recruited children and neonates (phase IV, open-label) from two different hospital settings (Alder Hey Children´s NHS Foundation Trust and the Liverpool Women´s Hospital). We had anecdotal evidence of clinical failures with teicoplanin therapy and had also observed "MIC creep" for CoNS often found at the breakpoint (4mg/L) (unpublished data). To further investigate the clinical pharmacology of teicoplanin in the paediatric population and to provide an insight into appropriate regimens for children, we performed the population PK study.

Three clinical population PK modelling analysis are presented in this thesis: children (n=39), neonates (n=18), both together (n=57), receiving teicoplanin in the inpatient clinical setting.

1) The population PK model in children > 1 month (n=39) revealed highly variable PK and was able to explain a 29% of variability in the population (Chapter 5). A standard 2-compartment PK model was developed, followed by structural models that incorporated weight. Weight was allowed to affect clearance (CL) using linear and allometric scaling terms. In this study, the linear model best accounted for the observed data and was subsequently chosen for Monte Carlo simulations. In addition, there was a much wider spread of drug exposure distribution (AUCs at steady state) when compared with adults. Thus, TDM should be performed routinely in children receiving teicoplanin.

Participants largely did not have renal impairment. The impact of reduced renal function on clearance and therefore dosing could not be assessed. We could not correlate drug exposure with effect/clinical outcomes in this study because there were only few documented infections.

2) The population PK model in neonates (n=18). The PK allometric model best accounted for the observed data and was able to explain a 25 % of PK variability in the population (Chapter 6). The simulated median $AUC_{96\text{-}120}$ was 302.3 mg*h/L, median C_{min} at 120 h was 12.9 mg/L. A 38.8% of patients attained a C_{min} >15 mg/L by 120h. Of the multiple covariates that were studied, only weight accounted for any portion of the observed PK variability. Incorporation of weight into structural PK models resulted in better fits and statistically more likely solutions. Of note, we could have equally reasonably related weight to clearance using linear or power scaling terms, despite the convention for using a scaling exponent of 0.75 (Anderson & Holford 2008; Germovsek et al. 2016). We could not demonstrate any relationship between teicoplanin clearance and PMA, estimated glomerular filtration rate (eGFR) or serum creatinine. This was somewhat surprising because teicoplanin is almost completely renally (98%) cleared by glomerular filtration (A. P. Wilson 2000). The absence of any relationship probably reflected the small sample size, as well as the relatively poor estimates of eGFR in neonates using current nomograms (Haycock-Schwartz) (Schwartz et al. 1984). This finding does call into question whether teicoplanin dosing should be adjusted on the basis of eGFR and further studies are required to specifically address this question.

This study was also too small to evaluate clinical exposure-response relationships. A Gram-positive pathogen was isolated in only 6/18 (33.3%) of patients. Hence, there was no opportunity to examine the relationship between the magnitude of any traditional pharmacodynamic indices (e.g. AUC:MIC) and outcome. Even in larger

datasets, the problem of culture negativity is frequently present. The use of CRP and a novel pharmacodynamic index (the AUC:EC50) was explored in this study. The rationale behind this ratio is that EC50 is an *in vivo* measure of drug potency and the AUC:EC50 is a measure of the exposure of drug relative to the potency of its effect. A major strength of this approach is that drug exposure targets are more individualised for a specific patient. The EC50 captures the impact of multiple variables on exposure-response relationships (e.g., resistance, high bacterial load, a persistent infective focus, biofilms and immune response). In contrast, when the measure of potency is the MIC alone, as for AUC:MIC, it is only the organism's characteristics that are considered. The model-Bayesian posterior estimates for EC50 ranged widely (0.6-18.7 mg/L) in the neonatal population, which again reflects highly variable pharmacodynamics. In this study, the AUC:EC50 predicted the terminal CRP levels after 5 days of therapy for a majority of patients.

3) The population PK model across childhood (n=57) was able to explain only a 28% of the variability in the population, confirming, on one hand, the high PK variability previously seen in neonates and children (Chapters 5 and 6), separately, and by other authors in similar paediatric populations (Lukas et al. 2004; Zhao et al. 2015a); and on the other hand, that a very high proportion of the inter-individual variability remains unexplained. Intra-individual day-to-day variability could represent a portion of the unexplained PK variability found, particularly in neonates and/or critically ill patients, nonetheless, measures were taken to decrease and control for this. Day-to-day changing serum creatinine and thus, eGFR values along the study were recorded to reflect the potential renal function´s changing pathophysiology. In addition, an inter-occasion variability (IOV) factor was incorporated into the whole dataset to account for

other changing physiological variables, other than renal, between the two study visits (before and after 4 days of therapy)(Karlsson & Sheiner 1993). Nonetheless, the final model showed there was little IOV on a population level, suggesting that this factor could be adequate for description but less helpful for dose-control.

In the covariate analysis, weight, as a surrogate of size, continues to be the most explanatory variable of teicoplanin´s behaviour in this model including neonates to older children, as in the previous population PK models of teicoplanin in paediatrics (Zhao et al. 2015b). It had an impact on both, clearance and volume of distribution (Ke) following an allometric scaling of factors (Anderson & Holford 2008). As for the renal function, we used the Schwartz-Haycock equation as the accepted standard to estimate GFR in children (Schwartz et al. 1984). In this study, eGFR was associated with teicoplanin clearance in both a linear and a power function manner, where GFR was the independent variable raised to a constant to be estimated (pw). Furthermore, the influence of growth and maturity in the renal function of children, was investigated with the patient´s age, indicating a similar power function association, as showed elsewhere (Heilbron, M. a Holliday, et al. 1991; Rhodin, Anderson, Peters, Coulthard, Wilkins, Cole, Chatelut, Grubb, Veal, Keir & Nick H. G. Holford 2009). On the other hand, a serum creatinine-based covariate accounting for the age (PNA/srCreat) was studied in the neonates and young infants < 3 months, where developmental changes occur in a more rapidly and pronounced way (Rhodin, Anderson, Peters, Coulthard, Wilkins, Cole, Chatelut, Grubb, Veal, Keir & Nick H. G. Holford 2009), and resulted in a better descriptor of teicoplanin elimination than the Schwartz equation in this sub-group of age. The usefulness of PNA/srCreat as a measure of renally eliminated drugs have been previously described in neonates receiving gentamicin and vancomycin (Neely & Floyd 2015; Yamada et al. 2015). This renal function estimator now needs to be further

validated in this population and with other drugs that depend extensively on renal elimination.

As discussed above, PK/PD models can combine available knowledge of PD, based on *in vitro*, animal or clinical data, with clinical PK. Because of the PK (and PD) variability in neonatal and paediatric patients, dosing simulations are required for different clinical covariates to propose dosing regimens suitable for individual patients. These dosing simulations can incorporate relevant patient characteristics known to affect PK, e.g. body size (weight) and organ function (eGFR). Attainment of PD targets can be predicted for different combinations of antimicrobial doses and pathogen susceptibilities, and optimised dosing regimens can be suggested for specific scenarios. Still, the predictions may be imperfect given the uncertainty in PK and PD present and should be validated in clinical studies. Furthermore, an individualised dosing and TDM approach can ensure optimal target attainment at the individual level and on real-time.

Population PK/PD modelling of clinical data

In the case of modelling the clinical data, the aim was defining the PK (and if possible PD) variability and relevant explanatory clinical covariates in the target population. This understanding allowed subsequent simulations for target attainment analysis and to be used as Bayesian prior information in the individualised treatment of similar patients receiving the drugs of study.

In the final study, a single teicoplanin population PK model for the three heterogeneous sub-populations across childhood (neonates, infants and older children up to 16 years old) was successfully developed with a non-parametric population modelling approach. This approach allowed increasing the sample size to 57 children,

which could further allow the investigation of the influence of covariates such as the eGFR and the age, whilst also exploring the major strength of Pmetrics for detecting unexpected sub-populations and outliers within the study population (Neely et al. 2012).

A comparison of both parametric (NONMEM) and non-parametric (Pmetrics) methodologies would be needed to complement findings on estimates for teicoplanin´s Cl and Vc and the impact of size and maturation across childhood. For modelling clinical data, a more consistent and standardised approach between both methodologies to model development, fitting and reporting would be optimal. This would make comparisons and meta-analyses amongst different models possible. This way a more efficient development of clinical PK/PD knowledge amongst a highly vulnerable population would be reached to ultimately help improving patient´s clinical outcomes.

8.4- INDIVIDUALISED THERAPY

In view of the high PK and PD variability in children and neonates, as well as the significant portion that results unexplained, a move towards individualised dosing is required. PK models coupled to a PD target can be used to design nomograms in which one or more patient characteristics that drive the PK variability are required to determine optimal dosing (Roberts et al. 2011). Dosing based on validated nomograms increase the probability of achieving PD targets as compared to conventional dosing (Cristallini et al. 2016). However, the significant amount of PK (and PD) variability that is not accounted for by covariates cannot be handled appropriately by nomograms. Bayesian calculations and forecasting of real patient´s clinical data are best suited to aid in the optimised use and truly individualised TDM of antimicrobials in general, and teicoplanin in particular (Roberts et al. 2014; Macdonald et al. 2008).

We used the model (prior past information) within a multiple-model Bayesian adaptive control algorithm. A multiple-model approach allows the use of multiple discrete individual models (one for each subject) as a discrete collection of points to find the most likely population parameter distribution. Each point (support point) contains a model parameter set of estimates and their associated individual probability. This algorithm was used to: 1) recompute the parameter distributions- based on the past experience in the general paediatric population- for an individual patient, and 2) to find the most likely balance of credibility between the population model and the new patient´s data, to predict future concentrations and calculate dosages to achieve target concentrations. This balance then can optimise teicoplanin therapy and inform individualised TDM in hospitalised neonates and children. In our study, early stage concentrations (first dosing interval plus a 48h trough), independently of steady-state conditions, allowed predicting the required dosage to achieve a desired recommended concentration target from day three of therapy.

The impact of intra-patient variability needs to be carefully taken into account. A bedside feedback control tool allows the continuous update of teicoplanin concentration-time data and patient´s covariate information to be re-entered into the software. This can account for real intra-patient variability along therapy. Nonetheless, rapid assessment of patient samples (i.e teicoplanin quantification) before the next dose is required to reduce the impact of PK variability and maximise the use of BestDose.

More recently, newer control algorithms, such as interacting multiple-model (IMM) analysis, could be best suited to account for the most unstable patients by letting parameter distributions change with each new data point (Macdonald et al. 2008).

8.5- FUTURE DIRECTIONS TO IMPROVE THE PK/PD TRANSLATIONAL PHARMACOLOGICAL APPROACH

From a clinical perspective, an improved development of the current PK/PD translational pharmacological approach is needed for: 1) the identification of optimal drug exposures in all sub-populations of paediatric patients and 2) the delivery of those optimal drug exposures to paediatric patients in a more individualised and controlled manner.

For the first requirement, pre-clinical PK/PD work needs to incorporate more correlation studies between drug exposures and the molecular characterisation of resistance development. Optimal drug exposures might be greater in the case of isolates exhibiting intrinsic survival advantages under prolonged drug pressure (i.e VISA isolates). Targeted *in vitro* HFIM studies would be best suited to undertake and characterize these exposure-effect complex relationships.

Importantly, clinical PK/PD studies linking those drug exposures with suppression of emergence of resistance in the target population of patients would also be needed. Currently, there are no published studies as such. These are required to cross-validate and place into context the magnitude of drug exposures (and PD indexes) deemed relevant from the pre-clinical models of infections. These studies are potentially complex to conduct and would require: 1) well identified patients in terms of clinical and microbiological characteristics. This needs the investigation of the bacterial density size (i.e DNA bacterial load) at the time of therapy initiation and at the infection site; 2) the sensitive detection of emergence of resistance mechanisms from the causative pathogens infecting recruited patients; and 3) measurement of the concentration-time course of the drug and linked inflammatory biomarkers at the site of infection.

For the second requirement, a PK/PD clinical population model from the target population would be developed, prospectively validated and used as prior information in the individualised PK-PD therapy software. This software ideally will use a dual-output Bayesian adaptive control algorithm able to: a) let the parameter distributions predictions change according to the variable pathophysiological changes in the patient and (i.e interacting multiple model algorithm) b) adapt dosing not only to achieving a population-established drug exposure target but also to reduce the patient-specific host-response biomarker ± bacterial load (i.e EC50) during the course of therapy.

8.6- FINAL CONCLUSIONS AND RECOMMENDATIONS

- The current strategy of using teicoplanin fixed population-based antibiotic regimens results in a wide range of drug exposures in neonates and children. A shift towards individualised dosing and TDM is required to: 1) maximise antimicrobial efficacy, 2) supress/minimize the emergence of resistance and 3) minimize potential drug-toxicity.
- Evidence-based PK/PD dosing regimens for specific patient populations and infection syndromes are fundamental to improve clinical outcomes in patients with severe infection.
- A population PK model of teicoplanin in children can be used as prior information to develop Bayesian adaptive control software to aid in the application of truly PK/PD based individualised therapy. The cost-effectiveness and feasibility of PK/PD-based individualised therapy in the real clinical context needs prospective evaluation.
- A standardised approach for existing PK/PD modelling methodologies to model development, fitting and reporting would be optimal. This will allow to maximise the utility and sharing of information obtained from clinical PK/PD studies in children.
- Clinical PK/PD studies need to be improved to provide maximally informative data from target populations. More PK-toxicodynamic studies to balance with the efficacy of higher PK/PD targets to combat the emergence of resistance are required. This is particular important in combination therapies and where there is limited toxicity clinical data from single dosing and from healthy adult volunteers.

- PK/PD information is currently difficult to obtain from patients, thus a pharmacological approach using pre-clinical, combined with clinical PK/PD information and mathematical and statistical modelling techniques will remain essential as a first step. Better experimental designs and tools are needed to determine the impact of factors such as, the human immune system and host-response, the protein binding and the bacterial burden into the antimicrobial exposure-response relationships in patients.

Chapter 9

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Chapter 10

Appendices

10.1- TEICOPLANIN PK (LIPSTIC) STUDY PROTOCOL V.03 05082013 (Table of Contents)

STUDY PROTOCOL:

Liverpool Pharmacokinetic / Pharmacodynamics Study of Teicoplanin in Children (LIPSTIC)

Version: 3.0

Sponsor Reference: 12/67/RE
REC Reference: 13/NW/0023
EudraCT: 2012-005738-12

Prepared by:

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Contents

10.2- CASE REPORT FORM FOR THE PK (LIPSTIC STUDY) V.04 072013

LIPSTIC

Liverpool Pharmacokinetic/Pharmacodynamics study

of Teicoplanin in Children

EudraCT No.: 2012-00538-12

Case Report Form

Version 4 (July, 2013)

Eligibility and Consent

1st two letters of patient name: |__||__|

1st three letters of patient surname: |__||__||__|

Centre number (LWFT: 01/ AHFT: 02): |__||__|

Inclusion number in the study: |__||__|

Date of inclusion: |__||__|/|__||__|/|__||__||__||__| (dd/mm/yyyy)

Date of birth: |__||__|/|__||__|/|__||__||__||__|

Inclusion criteria

	Yes	No
In-patients at AHFT or LWFT who are either: **-Neonates (24-39 weeks gestational age, 0-28 days)** **-Infants/Toddlers aged 1 to 24 months** **-Children aged 2 to 16 years**		
Who must be prescribed teicoplanin for suspected or confirmed Gram positive bacterial infection while in hospital, as part of their routine care.		
Provided parental informed consent		

Exclusion criteria

	Yes	No
Unlikely to survive 72 hours after recruitment (as judged by the clinical team caring for the patient)		

Person(s) with parental responsibility has consented to:

DNA sample ☐

Request a copy of the study results ☐

Consent form signed by the parents (or legal representative)

Date |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

1 copy given to parents yes ☐
original for research file yes ☐
1 copy medical notes yes ☐

Assent form signed by the patient (8-15 years old)
1 copy given to parents/patient yes ☐
original for research file yes ☐
1 copy medical notes yes ☐

Eligibility:
Patient prescribed teicoplanin and is now eligible signed ……
Date ……..
Record eligibility determined by a qualified person ☐

ONLY USE IN CASE OF WITHDRAWAL

Consent Withdrawn by Parent

Date of Withdrawn: |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

Name of Parent: **Date consent was withdrawn:**

Describe whether consent is withdrawn from the PK study, DNA or all. Confirm with parent if the data already collected may still be used.

……..

Name of Person that discussed withdrawal of consent with the parent ……..

Demographics

For all ages

Gender:

☐ Male ☐ Female

DOB	__/__/__ (dd/mm/yyyy)
Chronological age	……. years/months/weeks/days

For neonates and infants (and pre-term up to 3 years old):

	Write a number	
Post menstrual age* at birth (= Gestational age)	**___ ___ weeks ___ ___ days**	
Post menstrual age when starting the study	**___ ___ weeks ___ ___ days**	
Corrected age if preterm (up to 3 yrs)	**___ ___ weeks/months ___ ___ days** Developmental and recruitment purposes!!!	
Weight (g) (at study inclusion)	……. ……	
Height at study inclusion (cm)	……. ……	
ONLY FOR NEONATES (0-28 days)		
Apgar score at 5 minutes		
Apgar score at 10 minutes		
Birth weight (g)		
Height at birth (cm)		
	Yes	**No**
Surfactant		
Fetal growth restriction**		

***Post-menstrual age= Gestational age (time elapsed between first day of last menstruation and birth) + Chronological age (time elapsed after birth)**According to the local curve, birth weight below the 9th centile**

For older children: (at study inclusion)

Weight (Kg): **Height (cm):**

Indication of teicoplanin treatment (at start of treatment, tick where appropriate)

- Suspected infection (source not known) □
- Proven (microbiologically documented) Gram positive infection (source not known) □
- Suspected Central Venous Catheter (CVC) infection/ central line-associated bloodstream infection (CLABSI) □
- Proven Central Venous Catheter (CVC) infection/ CLABSI □
- Surgical-site infection (SSI) □
- Ventilator-associated pneumonia (VAP) □
- Other (specify) …… □

Summary of teicoplanin administration

Start of treatment |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

End of treatment |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

Has this patient been recruited into LIPSTIC before? YES/NO

Number of recruitment episode: |__|__|

Remember, if teicoplanin treatment stopped for a few days and then, re-started again, this would count as a different episode.

Study period

Each day from the start to the end of treatment						
Dose number	**Not administered***	**dose mg**	**Date** (dd/ mm/ yyyy)	**Start time of infusion/bolus**	**Stop time of infusion/bolus**	**Route of administration** (ie CVL/CVL single)
1			……/…../……			
2			……/…../……			
3			……/…../……			
4			……/…../……			
5			……/…../……			
6			……/…../……			
7			……/…../……			
8			……/…../……			
9			……/…../……			
10			……/…../……			
11			……/…../……			
12			……/…../……			
13			……/…../……			
14			……/…../……			
15			……/…../……			
16			……/…../……			
17			……/…../……			
18			……/…../……			
19			……/…../……			
20			……/…../……			
21			……/…../……			

*If teicoplanin was not administered code reason: a) no intravenous access; b) absent due to other procedure (e.g. theatre); c) other: describe.

**If the stop time has not been completed then check the nursing notes for any record of a delay in the infusion.

IMPORTANT!! If administered via a Broviac take into account the time needed for the drug to reach the blood. ***The time to record in the form is the time the drug reaches the blood.***

Co-administration

Record all antimicrobials/antifungals, cyclosporine, cisplatin, inotropes, antiepileptics, steroids, albumin and caffeine given with 72 h prior to first dose of study drug and through the last dose of study drug therapy.

Class	Drug name	Start date (day/ month/ year)	Stop date (day/ month/ year) or check if continuing
Antimicrobials			
Antifungals (ie Amphotericin B)			
Cisplatin			
Cyclosporine			
Inotropic drugs			
Steroids			
Antiepileptics			
Warfarin			
Albumin			
Furosemide			
Indomethacin			
Others:			

Caffeine Yes □ No □

Dose	Intravenous or oral	Date (day/ month/ year)	Start time of infusion or oral administration	Stop time of infusion or oral administration

The time to be recorded is the time the drug reaches the blood.

PK Blood Sample Planner and Record of Collection Times

1st Day (1st dose) of PK : |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

Last day of PK : |__|__|/|__|__|/|__|__|__|__| (dd/mm/yyyy)

Day of teicoplanin treatment: **a)** day 1☐ (first dose) or **b)** day 1/ 2 (subsequent doses) ☐

If b, was a pre-dose sample obtained? yes ☐ no ☐

then day 5☐ or day 6☐ or day 7☐

Was teicoplanin stopped early before day 5? yes ☐ no ☐

If yes, record reason: .....

24h post-last dose treatment (Wash-out period) ☐

PK Blood Samples

Stage 1 *This stage is for sample strategy definition and data interim analysis for accuracy. It will comprise the first 10 patients (including neonates). In this phase neonates will be only >1000 g.*

FOR NEONATES

Group	Weight		Sampling times per dosing interval on day 1 and day 5-7 of teicoplanin treatment			Maximum blood volume for all study samples*(ml per Kg body weight)
A	>1000g		T1	T3	T24	1.2 ml
	< 1000g	A1	T1		T24	0.8 ml
		A2	T1	T3		
		A3		T3	T24	
B	>1000g		T1	T6	T24	1.2 ml
	<1000g	B1	T1		T24	0.8 ml
		B2	T1	T3		
		B3		T3	T24	

PK Blood Samples

Stage 1 *This stage is for sample strategy definition and data interim analysis for accuracy. It will comprise the first 10 patients (including neonates > 1000 g).*

If neonates > 1000 g: record group A or B Group:…….

A: T1,T3,T24

B: T1,T6,T24 **DAY 1 (first dose)**

(If not possible, record accurately all timing of doses since first dose and once consent is granted, include subsequent doses)

Planned Sample Schedule	**Planned Sample Time**	**Actual Time sample collected**	**Date and time sample frozen at -20°C** *(must be within 24 hours of collection)*	**Sample source capillary /central venous/ arterial**	**Sample number**
T 1h					
T 3h					
T 6h					
T 24h					

DAY 5-7

Planned Sample Schedule	**Planned Sample Time**	**Actual Time sample collected**	**Date and time sample frozen at -20°C** *(must be within 24 hours of collection)*	**Sample source capillary /central venous/ arterial**	**Sample number**
T 1h					
T 3h					
T 6h					
T 24h					

PRE-DOSE SAMPLING (Only if first dose of teicoplanin is missed on day 1)

Planned Sample Schedule	**Actual Time sample collected**	**Date and time sample frozen at -20°C** *(must be within 24 hours of collection)*	**Sample source capillary /central venous/ arterial**	**Sample number**
T 0h (pre-dose sample)				

24 HOURS AFTER FINISHING TEICOPLAIN *In this first stage, if possible, a further sample at 24 hours of finishing the teicoplanin treatment will be taken for a **"wash-out" period** analysis.*

Planned Sample Schedule	**Planned Sample Time**	**Actual Time sample collected**	**Date and time sample frozen at -20°C** *(must be within 24 hours of collection)*	**Sample source capillary /central venous/ arterial**	**Sample number**
T W (+24 h)					

DNA SAMPLE (BLOOD SAMPLE) TO BE COLLECTED ONLY IN NON-TRANSFUSED PATIENTS

Confirm the patient has not received a blood transfusion: (circle) YES/ NO

Dates collected	**Date stored**	**Sample number**

Microbiological data-Culture results

Data reported **72 hours prior to start teicoplanin until day 10 after start of teicoplanin**

Include most recent Gram positive bacteria blood/ other sample (endotracheal tube secretion, surgical wound) culture. Teicoplanin MIC for positive Gram +ve samples needs to be required to Microbiology.

Samples for microbiological investigation should be stored only if Gram positive growth (Staphylococci, CoNs),

please tick if this was ensured □

Site of collection (code list)	**Date of collection**	**Organism** (state if no growth)	**Sensitivity/Resistance to teicoplanin**	**Date sample stored** (if applicable)	**Sample number if stored**	**MIC teicoplanin**

Code list: 1 Peripheral; 2 Central line; 3 Peritoneal fluid; 4 Endotracheal tube section; 5 Other (specify)

Laboratory results

If more than one blood sample has been taken in the day <u>record the first value available in the day</u> (if assessed for clinical care)

	72 hours prior to teicoplanin/ closest date Date:	**1st day of teicoplanin Date:**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**24h post-treatment Date:**
Full Blood Count									
Haemoglobin									
WBC									
Platelets									
Haematocrit									
Biochemistry									
Creatinine									
Albumin									
Total Bilirubin									
Conjugated Bilirubin									
AST/ALT									
ALK Phosphate									
Amylase									
CRP									
Blood Gas									
pH									
PCO2									
PO2									
Glucose									
Ionized Calcium									
Lactate									

Summary of clinical history

Cardiac Surgery	□
Oncology	□
General surgical	□
General medical	□

Date of current admission to hospital Unit/ward……

End of hospitalization (if available) ……..

Diagnosis at enrolment (including underlying conditions and surgery):

1.	**6.**
2.	**7.**
3.	**8.**
4.	**9.**
5.	**10.**

Diagnosis at end of therapy if different (including underlying conditions and surgery):

1.	**6.**
2.	**7.**
3.	**8.**
4.	**9.**
5.	**10.**

FOR ICU/HDU

Ventilation YES □ NO □

1st day ventilation …….

Ventilation	**Mode on Day 1 PK samples**	**Last Day PK samples**
HFO		
Pressure support /BIPAP		
CPAP		
Non-invasive CPAP		
Not ventilated		
ECMO		

Renal dyalisis: Yes□ No□ Peritoneal dyalisis Yes□ No□

Hemofiltration: Yes□ No□

Record transfusions during current admission

Include Blood Product: Packed Red Cells Fresh Frozen Plasma Cryo Human Albumin Solution Platelets

Date administered	Blood Product	Volume (ml)

FOR NEONATES, INFANTS AND PRE-TERM

PDA ☐ yes Surgery ☐ yes ☐ no If yes date:

☐ no Indomethacin ☐ yes ☐ no start date : …… end ……

Ibuprofen ☐ yes ☐ no start date:……..end……

Prostin ☐ yes ☐ no start date:……..end ……..

Intervention	1st Day PK Samples	Last Day PK Samples (day 5, 6, or 7)	24 h post-last dose of treatment PK sample
Nutrition: **Parenteral** **Breast Milk** **Formula**	 ☐ ☐ ☐	 ☐ ☐ ☐	 ☐ ☐ ☐
Gastroesophageal reflux	☐	☐	☐
Phototherapy	☐	☐	☐
Haemofiltration	☐	☐	☐
ECMO	☐	☐	☐
Cooling	☐	☐	☐

Outcome record from 1st dose until day 7 of treatment

Outcome	Details	Date
Surgery –any surgical procedure		
Necrotising Enterocolitis	**Surgical** ☐ **Medical** ☐	
Tolerability:– **state if teicoplanin was stopped due to a clinical concern**	**Reason stopped:**	
Fitting		
Intra ventricular Haemorrhage	**Grade I** ☐ **II** ☐ **III** ☐ **IV** ☐ **Grade not recorded** ☐	
Comments *Include any other information that may be relevant.* *Chronic clinical condition that may affect drug metabolism such as Downs, Cystic fibrosis, hydrops*		

Pharmacovigilance

During teicoplanin therapy, the study team will conduct a systematic daily review of patient records until 3 days after the last dose using electronic patient data system and consult carers and parents in order to identify adverse events.

The expected adverse events are described in the summary of product characteristics (SmPC) in the protocol appendix A. The aim of the study is not to report on safety. The Sponsor does not wish to receive reports about well-recognised adverse events during teicoplanin administration.

Serious Adverse Events Reactions or Suspected Serious Adverse Events or Reactions require expedited reporting <u>except for the Expected Serious Adverse Events in the group populations studied *(critically ill/pre-term neonates/oncologic patients)</u> at the discretion of Chief Investigator, treating physician.
Serious adverse events are classed as:

Result in death (excluded from expedited reporting in these patient groups)
Life-threatening
Requires hospitalisation or prolongation of existing hospitalization
Results in persistent or significant disability or incapacity

The Chief Investigator will be responsible for the reporting of the Suspected Unexpected Serious Adverse Reaction (SUSAR) to the National Competent Authority and Ethics Committee as appropriate.

Adverse event category:

Adverse event details:

Start date:

Stop date:

Action taken with study drug:

Outcome:

Intensity:

Severity:

General comments :

Adapted from TINN

Person completing the CRF (Date and signature):

10.3- PARENTAL CONSENT FORM FOR THE PK STUDY (LIPSTIC) V.02

Liverpool Women's NHS Foundation Trust

Parental Consent Form

Alder Hey Children's NHS Foundation Trust

LIPSTIC: Liverpool Pharmacokinectic/Pharmacodynamics Study on Teicoplanin in Children

A pharmacokinetic/pharmacodynamics study about teicoplanin, an antibiotic used to prevent and treat infections in neonates and children

Researchers: Chief Investigator Dr Stéphane Paulus, Principal Investigator Dr Mark Turner, Research fellow Dr Virginia Ramos Martin.

Name of baby/child: ……

	Please, Initials in Box
1. I confirm that I have read and understood the parent information leaflet and have had the opportunity to ask questions and these have been answered satisfactorily.	☐
2. I understand that my baby's/child's participation is voluntary and that we are free to withdraw at any time, without giving reason and without my baby's/child's medical care or legal rights being affected.	☐
3. I understand that sections of any of my baby's/child's medical notes may be looked at by the research team or the regulatory authorities for this research. I give permission for these individuals to have access to my baby's/child's records.	☐
4. I agree for my baby's/child´s samples of blood to be stored and used for current and future biological/genetic (DNA analysis) research projects relating to paediatric infections. I understand that these samples would be gifted and that no result on my baby's/ child's genes will be feedback to them or anyone else.	☐
5. I understand that my baby's/child's data may be analysed outside the trust (e.g. The University), protecting in any circumstance my baby's/child's confidentiality.	☐
6. I would like a summary of the results of the study when the research is completed.	☐

Name of parent: Relationship with the baby/child:

Signature: Date:

Researcher: Signature: Date:

Photocopy and give 1 copy to the parent, 1 for research file, original in medical notes.

10.4- PARENT INFORMATION LEAFLET V.02

Parent Information Leaflet

LIPSTIC: Liverpool Pharmacokinectic/Pharmacodynamics Study on Teicoplanin in Children

(A pharmacokinetic/pharmacodynamics study about teicoplanin, an antibiotic used to treat infections in neonates and children)

Thank you for taking time to read this leaflet. We would like to invite you to allow your baby/child to join this research study. Before you decide, please read this leaflet which explains the study. A member of the research team will then be available to go through the leaflet with you and answer any questions you might have. Please, feel free to discuss the study with family members or friends if you wish.

The purpose of this study is to evaluate how teicoplanin, an antibiotic, is handled by the body in children and neonates in order to update the drug dosage information for teicoplanin and provide an optimal dosage regimen. This drug has been used in children and neonates for a long time, when it was licensed (1989), extensive studies were not performed in the paediatric population and dosage regimen were mainly based in data from adults. This evaluation of an antimicrobial that is given as part of the routine clinical care, will help us to work out the most effective dose to treat infections, the time to wait between doses and the duration of the treatment.

Why has my baby/child been chosen?

You have been asked to consider taking part as your baby/child, during the time they are in hospital, they may require the antibiotic "teicoplanin" to prevent or treat infection. Your baby's/child's doctor (not the research team) may make a decision to start this antibiotic (it would be given through a drip as part of their clinical care).We would like to evaluate babies and children as soon as the treatment is started, therefore, we would like to ask for your consent at this stage.

We would like to include 54 children (babies and older children) for this study. Some of the children (neonates) will be treated at Liverpool Women's NHS foundation Trust and older children will be treated at Alder Hey Children's NHS Foundation Trust as per routine practice.

Does my baby/child have to take part?

No. It is up to you to decide whether your baby/child can take part. This decision will not affect the care your baby/child receives. If you do decide to take part and then change your mind, you are also free to withdraw at any time without giving a reason. You may request that any information already collected can also be withdrawn.

What will happen to my baby/child if she/he takes part in this drug level study?

- The research team will collect details from the baby's/child's medical notes To evaluate the level of the antibiotic in the baby's/child's blood we will collect 2-4 blood samples the first day of treatment and 2-4 samples at day 3-7 of treatment (only 2 samples at day 1 and at day 3-7 for babies weighting less than 1000 grams). When babies/children require this antibiotic, they often require other blood samples for their clinical care, whenever possible, we will plan to take these at the same time as the study sample to minimise disturbing your baby or child. The amount of blood required per sample will be of 0.2ml (4-6 drops for each sample).).
- In parallel, blood and other samples will be obtained routinely from patients for Microbiology investigation and when cultured positive for Gram positive bacteria, a determination of the microorganism susceptibility to teicoplanin (MIC) will be done and results collected for further analysis (pharmacodynamics). This information will be also used in the study.
- 'Genetics' –Some babies/children may respond better to this antibiotic than others, this could be because different genes have a better response than others. We would like to asses this by analysing DNA obtained from blood already taken for your baby's/child´s care. The genetic sample will only be used for the purpose of collecting information on your baby's/child´s response to infection or this medicine. If you choose to consent to the "Genetics sample" you will be asked to sign the appropriate box on the consent form.
- If your child is hospitalised in Alder Hey Children's NHS Foundation Trust, this blood sampling (for dug levels) will be performed, when possible, in the clinical research facility (CRF) to ensure maximal precision and quality. This is a paediatric dedicated facility with trained nurse staff and a paediatrician. If your child is receiving intensive care or it is not feasible to move to this facility, sampling will be made as usual. Parents and caregivers are welcome to accompany their children in both circumstances during sampling.

What are the possible benefits of taking part?

There are no direct benefits to your baby/child but the study may help the clinical team to know more about the best way to treat babies and children with this antibiotic in the future. Many studies of different treatments over the years have enabled us to develop the current care for babies and children. Studies involving children only provide expenses to families if they are required to travel, therefore, there will be no financial compensation.

Are there any side effects?

Teicoplanin would be given as part of the clinical care to treat a suspected or confirmed infection or to prevent an infection. Therefore, there are no additional side effects to this evaluation. Teicoplanin is a widely used drug that is already licensed for use in children and neonates. This study will check in more detail the effects of this drug by observing the effects of this treatment.

What are the disadvantages or risks of this study when taking part?

Taking blood samples may lead to some discomfort for the baby/child. Blood samples will be taken by staff experienced in taking blood in babies and children. The team will make an effort to minimise any distress with involvement of parents.

Some children will already have a line into a blood vessel to give fluid, other medicines or for monitoring, when possible, we will aim to take the sample from this line. In some cases, when this is not possible, then we may take a small amount of blood from a vein or a heel prick (the latter in babies). If we are unable to take the sample or if the baby/child is too distressed, then we will stop.

What happens after the study?

If you would like to receive a summary of the results of the study, this will be sent to you (if you agree, you will be asked to initial the appropriate box on the consent form).

What if something concerns me?

If you have any concern about the study you may speak to the researcher or the baby's/child's doctor or nurse who will discuss this with you and try to provide an answer. If you wish to complain, or have concerns about any aspect of the study, the National Health Service complaints mechanisms may be followed.

Will my taking part in this study be kept confidential?

If you do consent to the research, your baby's/child's medical records will be accessed by the research team or inspected by regulatory authorities to check that the study is being carried out correctly. Your baby's/child's name or other personal details will not be given to anyone else outside the hospital. Data may be analysed outside the Trust (e,g. at The University), however, all information will be managed anonymised and stored confidentially.

If you have agreed to a DNA sample this will be given a code so that it is confidential then stored in the Alder Hey Laboratory in Liverpool for a maximum of 10 years.

What will happen to the results of the research study?

The results will contribute to the information on the use of medicines for children and may be used to guide the dosage and administration of this drug for children and neonates. We will publish the results of the study in medical journals, present to other relevant staff and put a summary of the results onto the hospital website. The results will not be available until after the research has finished. If you would like us to send you a summary of the results please initial the appropriate section on the consent form.

Who is organising and funding this research?

The study is a MCRN (Medicines for Children Research Network)/LRN (Local Research Network) project and is supported by the National Institute of Health Research (NIHR) and the LRN.

Who has reviewed the study other than the research team?

The study has been reviewed by the UK National Research Ethics Service, The Medicines for Children LRN including doctors and nurses who are experts in the care of newborn babies and children. It has also been reviewed by the Research Governance Group at Liverpool Women's NHS Foundation Trust and Alder Hey Children's NHS Foundation Trust.

Contact for further information?

-Dr Stéphane Paulus, Infectious Diseases Consultant.

Alder Hey Children's Hospital

Telephone/email: stephane.paulus@alderhey.nhs.uk

-Dr Mark Turner, Consultant Neonatologist.

Liverpool Women's Hospital Telephone/email: mark.turner@liverpool.ac.uk

-Dr Virginia Ramos Martin. Clinical Research Fellow. Paediatrician.

Alder Hey Children's Hospital and Liverpool Women's Hospital.

Telephone/email: Research mobile number 24h available (07891410007))
/vrmartin@liverpool.ac.uk

The clinical team support this study, including the neonatal consultants or consultants at the Children's Hospital.

Thank you for reading this information sheet.

10.5- INFORMATION LEAFLET FOR CHILDREN < 8 YEARS OLD V.01, 8-12 YEARS OLD V.02 AND 12-15 YEARS OLD V.03.

LIPSTIC: Liverpool Pharmacokinectic/Pharmacodynamics Study on Teicoplanin in Children

INFORMATION FOR CHILDREN AGED UNDER 8 YEARS
(to be read by parent or guardian)

We have spoken to your mum and dad about taking some extra blood from you to look at one of the drugs you are currently receiving. This drug, called teicoplanin, is used to kill a bug that is causing an infection in your body. We are very interested in this drug and were wondering if you would mind helping us learn more about it.

We are asking all of the children having this drug if they will let us take some bloods from their long-line, catheter or by fingerprick.

These bloods will tell us how much of this drug is in the blood in your body.

If you are happy for us to take these bloods but then change your mind, it is OK to tell us to stop. We won't be upset or cross with you.

If you have any questions we will be very happy to talk to you about the study again if you want. Ask your mum and dad and they will contact us.

Liverpool Women's NHS Foundation Trust

Alder Hey Children's NHS Foundation Trust

INFORMATION FOR CHILDREN AGED 8-12 YEARS

(to be read by parent or guardian)

LIPSTIC: Liverpool Pharmacokinectic/Pharmacodynamics study on teicoplanin in children

You are having a drug called **teicoplanin** as part of your treatment and we were wondering if you would be happy to take part in a research study.

Please read this information sheet and talk about it with your mum and dad, your friends, and the doctors and nurses if you want. Ask us if there is anything that you are not sure about. Take plenty of time to decide whether or not you want to take part.

1. Why are you doing this study?

Teicoplanin has been used to treat infections for a long time. We know that when we give the same amount of drug to everybody, people will have very different levels of the drug in their blood, particularly children and adolescents. By taking your bloods we can measure the level of teicoplanin in your blood.

2. Why have I been chosen?

Everyone who is having teicoplanin in this hospital can take part in this study.

3. Do I have to take part?

No. It is up to you and your mum and dad to decide if you want to take part. If you take part but then change your mind that is okay and we will not be upset with you.

4. What will happen if I take part?

We will take 4 blood samples once you start your treatment with teicoplanin (day 1) and then another 4 samples at day 3 to 7 of your treatment. These blood samples will only be done during one course of your treatment. The amount of

blood we take is small (ie: 4 teaspoons) and will not do you any harm. In addition, some babies/children may respond better to this antibiotic than others, this could be because different genes have a better response than others. We would like to asses this by analysing DNA obtained from blood already taken as part of your care.

5. Confidentiality

All information collected about you for this study is confidential and will be stored securely in locked filing cabinets.

6. What if something goes wrong?

We are not expecting anything to go wrong but if there are any problems please tell us. If you are still unhappy in any way about the study your Mum and Dad can contact the hospital complaints department on your behalf.

7. What will happen to the results of the study?

The results of the study will be printed in medical magazines and may be used to change future treatment with teicoplanin. Your name will not be mentioned.

8. Who is organising the study?

The study is organised by a group of experts who work together to improve treatment for children with infections.

9. Who can I ask if I want to know more?

If you want to know anything else about this study please ask your doctor or the research nurses and they will be happy to talk to you again.

Thank you for reading this information sheet.

Contact:
Dr. Virginia Ramos Martin/ Dr. Stephane Paulus (phone via switchboard)
vrmartin@liv.ac.uk/ stephane.paulus@alderhey.nhs.uk

INFORMATION FOR CHILDREN AGED 12-15 YEARS

(to be read by parent or guardian)

LIPSTIC: Liverpool Pharmacokinectic/Pharmacodynamics study on teicoplanin in children

We are asking you whether or not you would like to take part in a research study involving the drug **teicoplanin**, which will be given to treat a suspected or confirmed bacterial infection.

Before you decide it is important for you to understand why the research is being done and what is involved. Please take time to read the following information carefully and discuss it with friends, relatives, doctors and nurses if you wish. Ask us if there is anything that is not clear or if you would like more information. Take the time to decide whether or not you wish to take part.

1. What is the purpose of the study?

Teicoplanin is an antibiotic that has been used in the treatment of a specific type of bacteria for many years. However, little is known about what happens to this drug after it enters the blood in children and neonates. We know from similar work with other drugs, that children differ in the way that they get rid of drugs from their blood. This study will measure how much drug is in the blood and see how and why this differs between children. This will help doctors to use this drug in the best way to treat infections in children.

2. Why have I been chosen?

Everyone who is going to receive teicoplanin while in hospital could take part in this study.

3. Do I have to take part?

No. Taking part in the study is entirely up to you. If you agree to take part and then later change your mind, you can stop at any time without giving a reason, this will not affect the treatment you receive.

4. Consent/ Assent

If you agree to take part in this study we will need your parents to sign a consent form and you could also sign the assent form. You will be given a copy of the assent form and this information sheet to keep.

If you decide at any point not to continue taking part in this study, you can do so without giving us any explanation.

5. What will happen if I take part?

A blood sample will be taken from your long line or drip if possible, just after the first dose of teicoplanin is given. Three further blood samples will be taken over the first day of teicoplanin treatment. In addition, another 3-4 samples will be taken at day 3 oto 7 of treatment. The amount of blood required per sample will be minimal and should not cause you any problems. The samples taken will allow us to measure levels of teicoplanin in the blood and look at variation between patients.

In parallel, blood and other samples, if necessary, such as samples from wound infections, will be obtained routinely from patients to see if the grow any bacteria in them causing the infection, and when finding growing bacteria, we will determine if that bacteria is susceptible to be killed at the amount of teicoplanin we are giving to treat the infection. In addition, some babies/children may respond better to this treatment than others, this could be because different genes have a better response than others. We would like to asses this by studying the DNA (the footprint that tells us how we are and makes everyone different) obtained from blood that has been already taken for your care.

6. Are there any disadvantages or risks involved in my participation in the study?

Taking blood samples may lead to some discomfort. Blood samples will be taken by staff experienced in taking blood in children. The team will make an effort to minimise any distress with involvement of parents.

Some children will already have a line into a blood vessel to give fluid, other medicines or for monitoring, when possible, we will aim to take the sample from this line. In some cases, when this is not possible, then we may take a small amount of blood from a vein. If we are unable to take the sample, then we will stop.

7. What are the possible benefits of taking part?

The information gained from the blood samples taken will not be of any help to you at the moment but may improve the way we use teicoplanin in the future in children.

8. Confidentiality

A study number will be given to you when you are first recruited onto the study. Only the first two letters of your first name and first three letters of your surname will be used.

Your blood samples and data will be all coded for analysis and they will be retained for the duration of the study. If you have agreed to a DNA sample this will be given a code as well so that it is confidential then stored in Alder Hey NHS Children´s Foundation Trust for a maximum of 10 years, which would allow us to do future research if it becomes appropriate.

9. What if something goes wrong or I am unhappy about any aspect of the study?

If you are unhappy about any aspect of the way you have been approached or treated during the course of this study, in the first instance please contact your consultant/ parent or guardian. If you are still unhappy, you can contact the hospital complaints department.

10. What will happen to the results of the study?

The study will run for approximately one year. Analysis will be carried out and the results published in medical journals, presented at international conferences and possibly used to modify future treatment. You will not be identified in any report or publication.

11. Who is organising and funding the research?

The study is a MCRN (Medicines for Children Research Network)/LRN (Local Research Network) project and is supported by the National Institute of Health Research (NIHR) and the LRN.

12. Contact for further information

If you require any further information please contact the consultant who is looking after you: **Dr Stephane Paulus, Infectious Diseases Consultant paediatrician. Telephone/email**: stephane.paulus@alderhey.nhs.uk
You also can contact: **Dr Virginia Ramos Martin. Clinical Research Fellow. Paediatrician. Telephone/email:** Research mobile number 24h available (via the switchboard) /vrmartin@liverpool.ac.uk

Thank you for reading this information sheet.

10.6- RESEARCH ETHICS COMMITTEE APPROVAL LETTER

NHS
Health Research Authority

National Research Ethics Service

NRES Committee North West - Liverpool East
HRA NRES Centre Manchester
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7832
Facsimile: 0161 625 7299

27 March 2013

Dr Stephane Paulus
Alder Hey Children's NHS Foundation Trust
Eaton Road
Liverpool
L12 2AP

Dear Dr Paulus

Study title:	**Liverpool Pharmacokinetic/pharmacodynamics study of teicoplanin in children (LIPSTIC).**
REC reference:	**13/NW/0023**
Protocol number:	**LIPSTIC.V.01**
EudraCT number:	**2012-005738-12**
IRAS project ID:	**120870**

Thank you for your letter of 14 February 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Miss Helen Penistone, nrescommittee.northwest-liverpooleast@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites listed in the application, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

The Committee specified the following additional condition:

- Blood samples stored, for future DNA analysis, within the Trust should be stored under the terms of the Human Tissue Authority Licence.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
REC application: 120870/396576/1/628		19 December 2012
Protocol	1.0	19 December 2012
Investigator CV: Stephane Christian Paulus		20 December 2012
Participant Information Sheet: Parent Information Leaflet	1	19 December 2012
Participant Consent Form	1.0	19 December 2012
Participant Information Sheet: for children aged 8-12 years	1.0	19 December 2012
Participant Information Sheet: for children aged under 8 years	1.0	19 December 2012
Response to Request for Further Information from Dr Paulus and Dr Ramos		14 February 2013
Participant Information Sheet: for children aged 12-15 years	02	04 February 2013
Participant Consent Form: Parental Consent Form	02	01 February 2013
Participant Consent Form: Assent Form for patients aged 8-15 years	1.0	18 January 2013

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/NW/0023	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

On behalf of
Mrs Glenys J Hunt
Chair

Email: nrescommittee.northwest-liverpooleast@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Dr Matthew Peak
Research and Development Office
Alder Hey Children's NHS Foundation Trust
Eaton Road
Liverpool
L12 2AP

Ms Dot Lambert,
Alder Hey Children's NHS Foundation Trust
Eaton Road
Liverpool
L12 2AP

10.7- MHRA APPROVAL LETTER TO AMENDMENT

Safeguarding public health

MHRA

Ms D Lambert
ALDER HEY CHILDREN'S NHS FOUNDATION TRUST
2ND FLOOR, RESEARCH AND DEVELOPMENT
EATON ROAD
LIVERPOOL
L12 2AP
UNITED KINGDOM

24/04/2013

Dear Ms D Lambert

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	21362/0003/001-0002
Eudract Number:	2012-005738-12
Product:	Targocid®
Protocol number:	LIPSTIC.V.01
Substantial Amendment Code Number:	Substantial amendment 01 - LIPSTIC Protocol version 2.0 19/03/2013

NOTICE OF ACCEPTANCE OF AMENDMENT

I am writing to inform you that the Licensing Authority accepts the proposed amendment to your clinical trial authorisation (CTA), received on 25/03/2013.

This amendment may therefore be made.

You are reminded that where it is appropriate, the Ethics Committee should also be notified of amendments.

Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare products Regulatory Agency
151 Buckingham Palace Road London SW1W 9SZ
T 0203 080 6000 www.mhra.gov.uk

An executive agency of the Department of Health

10.8- AHFT R&D APPROVAL LETTER

Alder Hey Children's NHS
NHS Foundation Trust
Research & Development
2nd Floor Mulberry House
Eaton Road, Liverpool
L12 2AP
0151 252 5673
Matthew.Peak@alderhey.nhs.uk
Dot.Lambert@alderhey.nhs.uk
Katherine.Jopson@alderhey.nhs.uk
Gavin.Soady@alderhey.nhs.uk
www.alderhey.com

Dr Stephane Paulus
Consultant in Infectious Diseases
Alder Hey Children's NHS Foundation Trust
Eaton Road
Liverpool
L12 2AP

29/04/2013

RE: A PK/PD study on Teicoplanin in children and neonates (LIPSTIC)
REC Ref: 13/NW/0023
R&D Ref: 12/67/RE

Dear Dr Paulus

Thank you for sending me the amended documentation for the above study. I am pleased to confirm that the changes made via SUBSTANTIAL amendment 01 Protocol v2.0 19/03/2013, which was given authorisation by the MHRA on 24th April 2013, are acceptable to the R&D office.

The documents approved for use are as follows:

Document	Version	Date
Protocol	2.0	19/03/2013

Yours sincerely

pp.

Dot Lambert
Research Business Unit Manager

10.9- TEICOPLANIN PK STUDY (LIPSTIC) SPONSOR LETTER

Alder Hey Children's NHS
NHS Foundation Trust

Alder Hey
Eaton Road
Liverpool
L12 2AP

Telephone: 0151 228 4811
www.alderhey.com

Research & Development Department
2nd Floor Mulberry House
Alder Road
L12 2AP

Tel: 0151 252 5673

Dr. Stephan Paulus
Consultant in Infectious Diseases
Alder Hey Children's NHS Foundation Trust

13th December 2012

Our ref: **12/67/RE**

Dear Dr. Paulus,

RE: A PK/PD study on teicoplanin in children and neonates

Under Research Governance framework for Health and Social Care, I write to confirm that Alder Hey Children's NHS Foundation Trust, will take responsibility as sponsor for the above named research study once Ethical and Trust approval has been granted.

With best wishes.

Yours sincerely,

Dot Lambert
Research and Development Manager

cc: Virginia Ramos Martin

INVESTOR IN PEOPLE